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Articles dealing with microscopic technic, or with the nature or uses of biological stains are suitable for publication in STAIN TECHNOLOGY. It is understood that articles submitted have not appeared previously elsewhere and that they will not be offered for simultaneous appearance in other publications without the consent of the editor of STAIN TECHNOLOGY.

Articles over two printed pages in length (i. e. about 800 words) must be preceded by a brief summary (100–400 words) which must be complete in itself and contain all the important facts brought out in the paper. When possible the abstract should summarize the new methods in such form that a reader can follow the directions without consulting the body of the paper.

All staining formulae must be definite and accurate. The batch or certification number of the stains actually used by the author in such formulae should be included for reference purposes. In case of uncertified stains, not designated by any batch number, one should give as much information as available, i. e. name of manufacturer, approximate date of purchase, etc.

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STAIN TECHNOLOGY

VOLUME 25 JANUARY, 1950 NUMBER 1

INTERIM REPORT OF THE COMMITTEE ON HISTOLOGIC MOUNTING MEDIA: RESINOUS MEDIA

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Received for publication June 1, 1949

On March 17, 1948, the Council of the American Association of Pathologists and Bacteriologists requested the Biological Stain Commission to undertake an examination of the currently available histologic mounting media. On March 25, 1948, Dr. H. J. Conn, President of the Biological Stain Commission, appointed Drs. R. D. Lillie, William F. Windle and Conway Zirkle as a committee to investigate mounting media. On April 16, 1949, the committee rendered a progress report to the Biological Stain Commission, and it was voted that the committee should forthwith submit its findings to date for publication. At the same meeting Dr. William F. Windle retired from the committee and was replaced by Dr. E. W. Dempsey.

The committee submits the following report:

ABSTRACT.—**The Fisher "Permount" naphthalene polymer, the Hartman Leddon "H.S.R." terpene polymer resin, a Monsanto polystyrene P-1, the Will Corporation "Diaphane" and "Green Diaphane", and the du Pont "Lucite" methyl methacrylate polymer were examined, and the possibility of use of some other plastics was also explored. The first 5 mentioned were tested for color preservation of a variety of stains in comparison with Canada balsam and Clarite X. From this point of view polystyrene, the Hartman Leddon "H.S.R." and the Fisher "Permount" resins were the most satisfactory, then the "Diaphanes". Both "Permount" and "H.S.R." show some yellowing. The H.S.R. with a melting point of 115°C, the Permount with 150°C. melting point, and the Polystyrene with a thermal denaturation point above 220°C. all excell Canada balsam**

in heat resistance. Trimethylbenzene, cymene and monoamylbenzene appear to be the best solvents for polystyrene. Mounts made in a solution of 20 g. polystyrene in 100 ml. trimethylbenzene can be packed flat slide to slide in 24 hours after mounting without sticking together.

This report is not intended to deprecate the use of other resinous mounting media which have not as yet been tested or compared with those mentioned herein.

Naphthene, Cycloparaffin, and Naphthalene Polymers

The naphthene or cycloparaffin polymers of 145–155°C. melting point, introduced by Groat (1939), deservedly attained wide usage. These resins, Nevillite V and Nevillite I, were later renamed, respectively, Clarite and Clarite X. Their refractive indices were given as 1.544 and 1.567 respectively. Unfortunately fires destroyed the manufacturing facilities and the Neville Company did not rebuild. Hence these resins are no longer available.

Permount, supplied by the Fisher Company, is described as a "water white", neutral resin with a melting point of about 150°C. The Fisher catalog refers to it as a naphthalene polymer with a refractive index higher than that of Canada balsam. It is available only as a light yellow toluene solution of stated 60% concentration which is quite fluid and spreads excellently, but is prone to form a surface scum before mounting can be completed. Owing to the amount and volatility of the solvent it may give air bubbles if evaporation is accelerated. The index of refraction of the commercial toluene solution is 1.5172 (Greco, 1950), its specific gravity 0.9312. On heating to 165°C. it loses about 37.5% of its weight and becomes a clear yellow liquid which solidifies around 150°C. into a brittle clear yellow solid. Assuming a 62.5% resin solution in toluene, Greco's figures indicate n_D 1.534 for the solid resin. The specific gravity was estimated at 0.9767 on the dried resin, and at 0.975 from solution data. Xylene solutions of the dried resin at 50, 60 and 70% by weight gave n_D = 1.513, 1.517 and 1.5209 at 20°C. Extrapolation suggests approximately 1.533 for the solid resin.

Terpene Polymers

The β-pinene or terpene ("piccolyte") polymers recommended by Wicks, Carruthers and Ritchie (1946) are not available from the manufacturer, the Pennsylvania Industrial Chemical Corp., in laboratory quantities. However, this company advises us that a resin of this class is being marketed by the Hartman Leddon Co. The latter company supplies both the dry resin and syrupy 60% xylene and toluene solutions, and now describes the resin as a terpene resin (May 1949 circular). When solutions are prepared in the laboratory from the dry resin, it is necessary to filter, in order to remove a certain amount of insoluble residue. Mounted preparations yellow somewhat with age. The resin possesses a moderately high melting point, 115°C. The refractive index of the dry resin calculated from the 60% xylene solution was advertised first at 1.5396, later at 1.540, both at 18°C.

Greco's figure of 1.5202 for the refractive index of the 60% xylene solution agrees well with the data from which the published refractive index was derived. This resin possesses a somewhat higher refractive index than the S-115 "piccolyte" resin quoted in Wicks, Carruthers and Ritchie's report (n_D 1.531).

Methyl Methacrylate Polymers

Methacrylate resins have been used by a number of workers with varying success. Skiles and Georgi (1937) reported no fading of stained bacterial smears after 11 months in butyl acetate solutions of "vinylite" and "pontalite" and in a xylene solution of "pontalite". "Pontalite" is a synonym of the methyl methacrylate resin "Lucite". Mounts were made both with and without cover glasses. Richards and Smith (1938) used solutions of lucite in dioxane, acetone, amylacetate and ethyl acetate. They reported fairly rapid fading of stains. Clearing was less satisfactory than in balsam or dammar. Dioxane-lucite set firmly in an hour, but later formed air spaces under the cover glasses on drying. The index of refraction of this methyl methacrylate was about the same as crown glass. E. C. Cole (1938) used duPont's Lucite in dioxane solution, and reported quick drying, lack of harmful effect on (unspecified) stains, lack of yellowing with age and an index of refraction of 1.50 to 1.52. This methyl methacrylate was also readily soluble in chloroform, but only slightly so in xylene. Davies (1940 cited from Wicks, Carruthers, and Ritchie) used a methyl methacrylate polymer called "Diakon" (n_D 1.503) and also had serious difficulty with loosening of cover glasses, though he did not complain of stain fading. Wicks, Carruthers and Ritchie found solutions of the methyl methacrylite polymer "Plexiglas" worthless.

In the current series of tests we found that only 2.58% yielded a thick syrupy solution in xylene. On drying, this solution gave rise to large air spaces under all of the cover glasses.

Following the suggestion inherent in the statement of Cole that methyl methacrylate was readily soluble in chloroform we prepared a solution of Lucite shavings in that solvent. Successive 100 g. portions of chloroform were added to 100 g. of the methyl methacrylate until a moderately thick rather viscous solution was attained. This required 700 g. solvent. The refractive index of this solution was 1.4589 at 20°C. (Greco, 1950). Preparations were mounted in it with some difficulty because of the high viscosity. On overnight drying at room temperature all preparations developed multiple large air bubbles. This experience accorded with our previous experience with chloroform Clarite solutions and with the use of benzene in mixtures with amylbenzene as a polystyrene solvent. It appears that highly volatile solvents are to be avoided, if the development of air bubbles is to be prevented.

The use of methyl methacrylate monomer was considered, and a sample was supplied us by the E. I. duPont de Nemours Co. The prescribed directions for removal of the inhibitor and the necessity for cold storage of the sensitized monomer were considered too formidable for routine biological laboratory use.

Isobutyl Methacrylate Polymers

O'Brien and Hance (1940) recommended a benzene or xylene solution of isobutyl methacrylate of unstated concentration which dried hard in 5 to 10 minutes. The

index of refraction was stated to be 1.477, and preparations appeared "somewhat more brilliant when viewed under the microscope than are specimens mounted in Clarite ($n_D = 1.544$)." Nothing was said about preservation of stains. Groat (1940) commented adversely on the report of O'Brien and Hance. He noted poor adherence to glass, rapid fading of certain stains and low softening and decomposition temperature. He apparently preferred media of high refractive index such as Clarite X and Clarite, and stated that the refractive index of the mounting medium should closely approach the refractive indices of stained tissue elements. The last point of view is at variance with that of a number of other writers, notably Lee (Microtomist's Vade Mecum, *4th Ed.*, Blakiston, Phila., 1896 and later editions), who stated "The *visibility* of minute structures is proportional to the *difference* between the refractive indices of the object and of the medium in which it is mounted."

Euparals and Diaphanes

Gilson's (1906) "Euparal" was praised highly by Lee (*8th Ed.*, 1924), especially for its low index of refraction (1.483). Gilson used a liquid mixture of camphor and phenyl salicylate of unstated proportions and n_D 1.53576, mixed with eucalyptol and paraldehyde as solvents for gum sandarac. The proportions were "communicated" to K. Holborn of Grübler's and have never been revealed. A secret "essence" for thinning was also available from the same company. A green variant "Euparal vert" containing a little of a copper salt conserved hematoxylin stains better. It was possible to mount in these media directly from alcohol of 70 to 100%. The colorless variant was recommended especially for Romanovsky stains and anilin dyes generally (Lee). In his 8th and 9th editions (1924, 1928), Lee recorded that Euparal was available from a British supply house, but that statement had disappeared in the 10th edition (1937). Romeis (1932) states that, on hardening, the index of refraction rose from Gilson's stated 1.483 to 1.535, which is essentially the same as Canada Balsam. Coulston (1941) reported on the Will Corporation's similar preparations "Diaphane" and "Green Diaphane". He found the low refractive index (1.483 for both variants) highly advantageous as compared with Canada balsam (n_D 1.535) and still more so in comparison with the cycloparaffins Clarite (n_D 1.544) and Clarite X (n_D 1.567) introduced by Groat (1939). The latter (1940) considered the high refractive indices of his media highly advantageous, especially in comparison with isobutyl methacrylate (n_D 1.477). Coulston completed the dehydration and mounting with a sequence of (1) 95% alcohol, (2) a mixture of 30 ml. 95% alcohol, 30 ml. acetone and 5ml. diaphane, (3) a mixture of 55 ml. acetone, alcohol and 10 ml. diaphane, and (4) pure diaphane.

A supply of these resins, "Diaphane" and "Green Diaphane" was furnished us by the Will Corporation. Refractive indices at 20°C. were slightly lower than the advertised 1.483: 1.4777 and 1.4792 for the colorless and green variants respectively (Greco, 1950). The resins have so far been quite good in preservation of azure-eosin stains, though somewhat inferior to Clarite X, Harleco synthetic and Fisher permount resins. They possess a strong aromatic odor suggesting the presence of both camphor and eucalyptol.

Kriston

A chlorinated allyl ester polymer called Kriston was supplied as monomer by the B. F. Goodrich Chemical Co. The polymer is described as resistant to alcohol, acids, alkalies and fat solvents. The monomer requires the use of a catalyst such as benzoyl peroxide,

and hence the substance was not deemed suitable for histologic purposes.

Polystyrenes

Kirkpatrick and Lendrum (1939) compared various mounting media: a cedar-oil-Canada-balsam solution, xylene colophonium, xylene dammar, cedar oil dammar, Yucatan gum elemi, "Teglac 161", Bakelite resin R-254 and the polystyrenes "Distrene 60, 80 and 120". Of these they preferred first "Distrene 80" and second "Teglac 161". "Distrene 80" in xylene showed very marked retraction under the cover glasses. Addition of tricresyl phosphate as a plasticizer prevented the retraction: tricresyl phosphate, 7.5 ml.; xylene, 40 ml.; "distrene 80", 10 g. This mixture was designated "DPX" and is probably the same as the "Depex" sold by G. T. Gurr in Britain. Smears stained by Leishman's stain, by Gram's method, by acridine red and by Malachite green retained their color 6 months in "DPX", while controls faded in 3 months. Gelatin impregnated with indicator promptly became acid when mounted in balsam, slightly so in "Teglac 161", and showed no change in the polystyrene mixture DPX. In a second report Kirkpatrick and Lendrum (1941) substituted 5 ml. dibutylphthalate with 35 ml. xylene and 10 g. "Distrene 80" for the DPX formula, noting equal preservation of the same stains. They noted crystallization of paraffin in polystyrene mounts, but not in balsam mounts, when the same xylene was used for clearing that had been employed for deparaffinizing sections.

Fleming (1943) complained of bad crazing of mounts in a polystyrene of 1.60 refractive index, which he had synthesized from styrene by heating on a water bath with a trace of benzoyl peroxide to solidity. He does not state what solvent was used.

Wicks, Carruthers and Ritchie (1946) claimed fair results with a polystyrene of $n_D = 1.59–1.61$ obtained from the Dow Chemical Co. The polymer was water white and of low acidity. With xylene solutions "adhesion to glass" was very poor, but was greatly improved by addition of 5–10% of dibutylphthalate.

We obtained a quantity of the polystyrene "Lustron", L–2020, formula PI, from the Monsanto Chemical Co.

Lustrons are described as soluble in aromatic hydrocarbons, chlorinated aliphatic hydrocarbons, esters, certain ketones and some tarpenes. They resist aqueous salts, acids and alkalies, water, lower aliphatic alcohols, and glycols. Lustrons undergo fairly rapid oxidation at temperatures over 220°C. Their refractive index is given at 1.59, specific gravity at 1.054, light transmission at 89–90%. Early experiments indicated that a 30% xylene solution was too viscous for satisfactory routine use. Xylene solutions at 15–20 g. per 100 ml. engendered large air bubbles on drying. Solutions at 18 g. per 100 ml. in equal volumes of commercial monoamylbenzene and xylene were very satisfactory, if slightly more of the resin solution was used than had been customary with Clarite. Similar mixtures of benzene and amylbenzene as solvent gave rise to large air bubbles. The amylbenzene used was probably largely secondary, with boiling point at about 189°C. and refractive index of 1.4894 at 20°C. As the odor of the amylbenzene was fairly strong with freshly mounted slides and disagreeable to a number of workers, further search for a suitable aryl hydrocarbon solvent was made after the amylbenzene xylene solution had proven very satisfactory in routine practice. Solutions at 18 g. per 100 ml. in p-cymene and in technical trimethylbenzene were satisfactory in regard to non-production of air spaces. Preparations

were tested at 58°C. in 10mm. Hg pressure as well as at room temperature (22–28°C.) and atmospheric pressure. Trimethylbenzene (boiling point 165–176°C., n_D 1.4931) was finally selected as having a more pleasant odor than amylbenzene and a lower cost than p-cymene (B.P. 176–177°, n_D 1.4866). Solutions of polystyrene L2020, at 18, 20, and 25 g. per 100 ml. trimethylbenzene, have been used routinely and prove quite satisfactory. Cover glasses become very difficult to move in an hour after mounting and slides may be packed face to face or face to back in drawers on the day following mounting, and they do not stick to each other. No thick labels to prevent contact of the cover glass with the next slide are required.

Refractive indices at 20°C. were determined for a number of solutions of polystyrene L2020. At 18 g. per 100 ml. of equal volumes of xylene and monoamyl benzene the refractive index is 1.5092 at 20°C., at the same concentration in trimethylbenzene it is 1.5117, at 20 g. per 100 ml. in trimethylbenzene 1.5145, at 25 g. per 100 ml. in the same 1.5188, at 18 g. per 100 ml. in monoamylbenzene 1.5050, while at the same concentration in p-cymene it is 1.5046 (Greco, 1950).

In 2 months an 18 g. per 100 ml. solution in trimethylbenzene loosely covered with gauze evaporated to about 86% of its previous volume, remaining perfectly fluid, with no surface scum, and was still not too viscous for use. The evaporation down to 86% of former volume corresponds to an increase to about 21.5 g. polystyrene per 100 ml. of trimethylbenzene, presuming the loss of volume to be all solvent. This relatively small increase in concentration after 2 months evaporation would seem to indicate that thinning of the solution should seldom be necessary.

Comparative Fading Tests

Several series of comparative fading tests were made. The first was a small series of sections stained by the azure A, eosin B technic (Lillie, 1948, p.84) and mounted in a xylene amylbenzene solution of polystyrene (14:10:6 by weight) and in a xylene solution of the Hartman Leddon synthetic resin. These were exposed to a 60 watt blue glass tungsten filament bulb at 20 cm. for 18 days, and then to a 100 watt bulb for a further 16 days. There was no evident fading in either series.

In the second series four mounting media were tried. A thick syrupy xylene-lucite solution (2.58%) formed very large air spaces very promptly after mounting. Two solutions of polystyrene at 18 g. per 100 ml. of solvent were used: xylene 50; amylbenzene 50 or 40; alphabromonaphthalene 0 or 10. Refractive indices were respectively 1.510 and 1.523. The fourth mounting medium was a syrupy xylene solution of Canada balsam. Sections were stained by the same azure A, eosin B technic as before, by the iron hematoxylin Van Gieson picro-acid fuchsin technic (Lillie, 1948, p. 190–1), by the acetic acid ferrocyanide and safranin technic for hemosiderin (ibid, p. 124) and the pH4 acetate-buffered thionin for chromophil substance of nerve cells.

Stained sections were exposed continuously to the light of a 100

watt blue glass tungsten filament lamp at 20 cm. distance for 5½ months. The Prussian blue reaction was largely lost in 2 weeks in Canada balsam, but perfectly preserved after 5½ months in the two polystyrene media. Thionin staining of tigroid (chromophil) substance of anterior horn cells was much weakened in balsam, well preserved in the polystyrene media. Red staining of collagen in Van Gieson preparations was weakened or lost in Canada balsam, well preserved in the two polystyrene media. With the azure A, eosin B stain, cartilage was deep blue violet after 5½ months in polystyrene, blue in the balsam mount. Staining of tigroid was reduced or abolished in balsam mounts, well preserved to reduce in the polystyrene mounts, and better preserved in the plain amylbenzene xylene mixture than in the α-bromonaphthalene variant. The strong cytoplasmic basophilia of pancreatic acini and peptic gland chief cells was well preserved in the polystyrene mounts, considerably impaired in the balsam mounts.

A considerable group of routine sections of mammalian intestine mounted in the 18 g. polystyrene per 100 ml. of equal volumes of amylbenzene and xylene medium were laid aside for 3–5 months before examination. The following stains were found to be satisfactorily preserved: 1. Azure A, eosin B (Lillie, 1948, p. 84); 2. Iron hematoxylin, fast green FCF, safranin O sequence (ibid, p. 149); 3. Schiff reactions after periodic acid (p. 188), chromic acid (p. 143) and potassium permanganate (p. 105), silver and ferric ferricyanide methods for argentaffin cells (Laskey and Greco, 1948, Burtner and Lillie, 1949).

In a third electric light fading test 96 slides were exposed continuously under two 100-watt blue glass lamps set at 20 cm. above the table. Trays containing 24 slides each were used and the positions of these trays were rotated 3 times a week. Six mounting media were employed; Fisher Permount (60% in toluene) (naphthalene polymer); polystyrene L2020 18 g. in 100 ml. trimethylbenzene, Will Corp.'s Diaphane and Green Diaphane; Hartman Leddon synthetic (β-pinene polymer) resin 60% in xylene; Clarite X (cycloparaffin polymer) 60% in xylene. Sections were stained by azure A, eosin B as before, by acetic ferrocyanide for iron as before, by iron-hematoxylin-Van Gieson as before, by a Masson trichrome stain, by hemalum and eosin, and by the Burtner-Lillie (1949) argentaffin cell method.

In this series fading of azure eosin and ferrocyanide preparations was advanced in 10 weeks. In general nuclear and cytoplasmic basophilia showed the greatest losses in the two diaphanes, next in the Fisher and Hartman Leddon resins and least in Clarite X and polystyrene, but differences between them, especially the last 4, were slight. Eosinophilia was best preserved with the Fisher cycloparaffin polymer. The basophilia of cartilage was lost somewhat more rapidly in the diaphanes, least rapidly in Clarite X and polystyrene, but again differences were not great. Preservation of metachromatic staining of vertebral nucleus pulposus was slightly better in polystyrene and poorest in the diaphanes. Again differences were small. Nissl staining persisted almost equally well in all six

media. Here green diaphane and polystyrene seemed to have a slight advantage.

Prussian blue reactions were perhaps least impaired in Diaphane, Hartman Leddon β-pinene polymer and Fisher "naphthalene" polymer, but preservation throughout was much worse than on the previous series.

At ten weeks Van Gieson stains were exhibiting some fading of the acid fuchsin in the two diaphane resins. The four synthetic resins gave generally good preservation of the red elements. Masson trichrome stains were generally little altered in any of the six media. Similarly hemalum eosin stains and Burtner-Lillie argentaffin cell stains were well preserved. Observation of these preparations is being continued.

Hyrax, Naphrax, Etc.

Hanna's (1930) Hyrax was made from naphthalene by an unrevealed process. It was quite thermostable, remaining clear at temperatures high enough to scorch gelatin. Its melting point was not stated. Its refractive index was 1.82248, and its principal use was for the mounting of unstained diatoms. It did not fade "common stains used in biology". It was slowly turned yellow by sunlight. It was soluble in aromatic hydrocarbons.

Fleming's (1943) "Naphrax" was synthesized from formaldehyde and naphthalene and plasticized with dibutyl phthalate. It was soluble in aromatic hydrocarbons and possessed a refractive index of 1.76 to 1.80. This resin was used alone in toluene solution, or mixed with a polystyrene of 1.6 n_D which was polymerized from styrene by heating with benzoyl peroxide.

There remain to mention the German resin Caedax, the British resins Xam, and Depex, samples of which are being procured for trial, and Mahady's Micromount, samples of which have just been obtained.

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REFRACTIVE INDICES OF CURRENTLY USED MOUNTING MEDIA

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In connection with studies on mounting media by a committee of the Biological Stain Commission[1], the indices of refraction of many media and solvents were taken.

The indices were determined by using an Abbe refractometer made by Bausch and Lomb Optical Company. This instrument reads the refractive index directly and requires only a drop of the sample. Three measurements per sample were made and the average recorded Since the indices were determined at various temperatures, the readings were converted to 20°C. for convenience of comparison[2]. Several well known organic solutions were measured as controls.

As many of the resinous and water soluble media have not been recorded previously, it is thought worth while to present them in tabular form. (See next page.)

Some of the indices recorded in the table vary slightly from those previously published. In these reports the temperature at which the index was determined and the strength of the solution sometimes were not given.

[1]Lillie, R. D., Windle, W. F., Zirkle, C. 1950. Interim report of the Committee on histologic Mounting Media: Resinous media. Stain Techn., **25**, 1–10.

[2]Lange, N. A. 1944. Handbook of Chemistry. *5th Ed.* Handbook Publishers, Sandusky, O. *See p. 932.* (The index of refraction of a liquid decreases with the rise in temperature and this thermal variation differs with various substances but is approximately 0.0004 per 1°C.)

Refractive Indices of Resinous Media

Substance	Company	Lot No.	Per Cent of Substance by Weight	n_D	Temperature	Conversion to 20°C.
Balsam, Canada (neutral in xylene)	Biosol Products	123	"syrupy"	1.5330	18°C.	1.5322
Balsam, Canada (60:40 in xylene)	Coleman & Bell	1437	60%	1.5268	28°C.	1.5300
Curtis salicylic acid balsam	N.I.H.		"syrupy"	1.5197	26°C.	1.5221
Clarite X (60:40 in xylene)	Neville Co.		60%	1.5360	18°C.	1.5352
Damar Resin (60:40 in xylene)	McKesson & Robbins	1678c	60%	1.5293	26°C.	1.5317
Diaphane (colorless)	Will Corp.		"syrupy"	1.4745	28°C.	1.4777
Diaphane (green)	Will Corp.		"syrupy"	1.4762	28°C.	1.4794
Harleco (β-pinene) resin (60:40 in xylene)	Harleco	7885	60%	1.5178	26°C.	1.5202
Lucite (in xylene)	Dupont Co.	H 7500	2.58%	1.4970	18°C.	1.4962
Lucite (10:70 in chloroform)	Dupont Co.	H 7500	12.5%	1.4565	26°C.	1.4589
Mahady Micro-Mount EFM	E. F. Mahady Co.	6160	"syrupy"	1.4890	27°C.	1.4918
Mahady Micro-Mount (solid resin—calc.)	E. F. Mahady Co.	6160		1.4843	29°C.	1.4879
Permount (62.5:37.5 in toluene)	Eimer & Amend	118	62.5%	1.5140	28°C.	1.5172
Permount (14:6 in xylene)	Eimer & Amend	118	70%	1.5189	25°C.	1.5209
Permount (12:8 in xylene)	Eimer & Amend	118	60%	1.5146	26°C.	1.5170
Permount (10:10 in xylene)	Eimer & Amend	118	50%	1.5106	28°C.	1.5130
Permount (solid resin—calculated)	Eimer & Amend	118		1.5340	29°C.	1.5376
Polystyrene, 18 g. in 100 ml. sec. amyl benzene	Monsanto Chemical Co.	L2020	17.3%	1.5050	26°C.	1.5074
Polystyrene, sec. amyl benzene-xylene (20:33:47)	Monsanto Chemical Co.	L2020	20%	1.5100	18°C.	1.5092
Polystyrene, 18 g. in 100 ml. p-cymene	Monsanto Chemical Co.	L2020	17.3%	1.5023	26°C.	1.5047
Polystyrene, 18 g. in 100 ml. trimethylbenzenes	Monsanto Chemical Co.	L2020	17.3%	1.5093	26°C.	1.5117
Polystyrene (20:80 in xylene)	Monsanto Chemical Co.	L2020	20%	1.5151	27°C.	1.5179
Polystyrene (50:50 in xylene)	Monsanto Chemical Co.	L2020	50%	1.5350	27°C.	1.5378
Polystyrene (solid resin—calculated)	Monsanto Chemical Co.	L2020		1.6243	29°C.	1.6279

Refractive Indices of Solvents

Sec. amyl benzene (Tech.)	Eastman	T4943		1.4860	18°C.	1.4852
p-cymene	Eastman	83		1.4868	30°C.	1.4908
Glycerol	Baker	12947	95%	1.4650	26°C.	1.4674
Toluene (CP)	Baker	101547		1.4928	27°C.	1.4956
Trimethylbenzenes (Tech.)	Eastman	T2549		1.4931	27°C.	1.4959
Xylenes (Tech.)	Baker	121643		1.4950	28°C.	1.4982

Refractive Indices of Water Soluble Media

Abopon	Glyco Products	F1157		1.4380	18°C.	1.4372
Apathy-Kasarinoff	N. I. H.			1.4405	35°C.	1.4465
Apathy-Lillie	N. I. H.			1.4165	26°C.	1.4189
Farrant's glycerol gum arabic	N. I. H.			1.4393	26°C.	1.4417
Fructose syrup	N. I. H.		48.6%	1.4338	26°C.	1.4362
Glycerol jelly (Kaiser's)	N. I. H.			1.4285	37°C.	1.4353
Highman's potassium acetate gum arabic syrup	N. I. H.			1.4205	26°C.	1.4229
Highman's salt gum arabic syrup	N. I. H.			1.4233	26°C.	1.4257
Lillie-Laskey diethylene glycol syrup, gum arabic	N. I. H.			1.4360	26°C.	1.4384

MAKING AUTORADIOGRAPHS OF INDIVIDUAL BLOOD CELLS[1]

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ABSTRACT.—**Single blood cell autoradiographs were made by smearing blood, diluted with serum, directly upon the emulsion of an Eastman NTB plate. After exposure, the plates were developed in D19. A 10% sodium sulfate solution was used for the photographic fixing to prevent lysis of the cells. After staining with Wright's stain the cells and autographs could be examined simultaneously under the microscope.**

INTRODUCTION

Since Altman, et al.[2] showed that the alpha-carbon atom of glycine labeled with C^{14} is incorporated into the hemin and globin moieties of hemoglobin, it was believed that the C^{14} incorporated into blood cells could be demonstrated by means of autoradiographs. A technic for making autoradiographs of individual blood cells containing C^{14} was developed, and data obtained by means of this technic have been published elsewhere[3]. In Fig. 1 are given some examples of single blood cell autoradiographs. The silver grains concentrated about the blood cells and constituting the autoradiographs were produced by the action of beta particles on the silver bromide grains of a photographic emulsion. These beta particles emanated from the C^{14} contained within the cells.

Comments on certain of the problems and difficulties related to the production of such autoradiographs are presented below, following the outline of the technic itself.

The conditions for photographic processing given below in the technic are those which have been used for Eastman Kodak NTB experimental plates with an emulsion thickness of 6 μ. Although the technic as outlined contains certain details of a specific nature, it

[1]This paper is based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

[2]Altman, K. I., Casarett, G. W., Masters, R. E., Noonan, T. R., and Salomon, K. 1948. Hemoglobin synthesis from glycine labeled with radioactive carbon in its a-carbon atom. Fed. Proc., **7**, 2; J. Biol. Chem., **176**, 319.

[3]Boyd, G. A., Casarett, G. W., Altman, K. I., Noonan, T. R., and Salomon, K. 1948. Autoradiographs of C^{14} incorporated in individual blood cells. Science, **108**, 529.

is offered as a guide and as an indication of the general approach in the making of autoradiographs of blood cells and other individual cells containing any radioactive element.

Technic

1. Gently mix the freshly obtained blood in a watch glass with a small amount of 1% oxalate solution to prevent clotting and shrinking. The ratio by weight of potassium oxalate to ammonium oxalate in the 1% solution was 1:1.5.

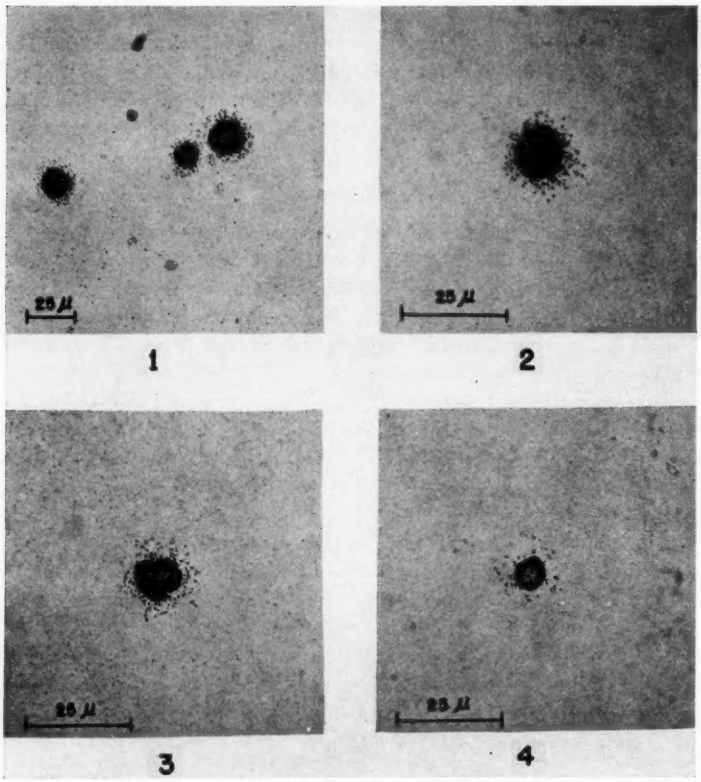

Fig. 1.—Autoradiographs of C^{14} incorporated in individual blood cells:
(1) Field of blood smear illustrating non-uniform distribution of silver grains and concentration of grains around certain cells (× 390).
(2) Lymphocyte (× 840).
(3) Polymorphonuclear leucocyte (×840).
(4) Erythrocyte (×840).
Reprinted, by permission, from Science, **108**, 529 (1948).

2. Dilute the oxalated blood with fresh, clear serum, to obtain the

desired cellular concentration and distribution. Stir gently, removing all large particles and clumps of cells.

3. Observing the usual darkroom precaution for NTB plates, smear a drop of the cell suspension directly on the emulsion by pushing forward a clean glass slide with the cell suspension pulled along behind it. With an angle of about 20° between the glass slide and the emulsion and only the weight of the slide pressing on the emulsion, scratches will not be produced in smearing.

4. Dry smears in air.

5. Fix the cells by flooding the plate with absolute methyl alcohol for two minutes. Pour off the alcohol and dry the smears in air.

6. Seal the plate, in the presence of calcium chloride or some similar desiccant, in a light-tight box, and store in a deep-freeze or refrigerator for the exposure period.

7. After exposure, develop the plate in Eastman Kodak D-19 developer for 2 minutes at 20°C., and rinse in tap water for ½ minute.

8. Fix the plate in freshly made 10% sodium thiosulfate solution until clear only, then wash immediately in tap water for 20 to 30 minutes. Dry in air.

9. Stain, as with ordinary blood smears. Only Wright's stain has been used to date in this laboratory.

10. Dry in air, mount in a suitable medium, and add coverslip.

Comments

The making of autoradiographs of cells containing C^{14} involves first the placing of the cells in close relation to a photographic emulsion sensitive to the particles emanating from the cells. The cells must then be fixed and the emulsion left to expose for a suitable period of time in a light-tight container. After the exposure period the photographic plate is developed and fixed, and the cells are stained.

Beta particles from an atomic nucleus within a cell can penetrate a photographic emulsion for several micra. Therefore, unless the cells are widely separated the resultant autoradiographs may overlap and become closely related to cells not actually containing the radioactive material which caused them. The gelatin contained in photographic emulsions rapidly imbibes fluid and thereby presents difficulty in making blood smears with suitable separation of cells. This problem may be solved by diluting the blood with serum before smearing. Dog serum was the serum of our choice because it is easily obtained in quantity and in our experience does not affect rat blood cells in ways deleterious to the aims of our present experiments.

It is difficult to predict closely a suitable exposure time, since this depends on the emulsion sensitivity, the isotope concentration in individual cells, and also on the aim of the experiment, which dictates

the type of autoradiograph necessary. In order to determine the optimal exposure time for a particular experiment, a number of exposures should be started simultaneously and developed serially in time until the desired results are obtained. In experiments involved in the technic outlined in this paper, an exposure time of 67 days produced satisfactory results with blood removed from a 120 gram rat 25 hours after intraperitoneal injection of 3 microcuries of glycine containing C^{14} in the alpha-carbon atom (specific activity 1.83 μc/mg.).

Since the blood cells are taken along with the photographic emulsion through the photographic developing and fixing solutions before staining, it is important that the concentrations of these solutions, and the lengths of time the plates remain in the solutions, be optimal for the maintenance of normal staining properties and morphological characteristics of the cells. The type of photographic emulsion used would exert some influence with regard to these factors. The photographic-fixing step is the most critical in the technic. Two variables which influence fixing, viz., the nature of the silver bromide grains and the emulsion thickness, can be held constant by the manufacturer for all plates made from any one emulsion batch. This was true for our experiments, in which Eastman Kodak NTB experimental plates, with an emulsion thickness of 6 μ, were used. These plates have extremely low "background fog" and, of all plates tested, are the most sensitive for their grain size to beta particles[4]. Variations in photographic processing may be necessary for other emulsions. For example, thicker emulsions may require greater fixing and washing times.

Sodium thiosulfate concentrations from 1% to 35% were tested. The 35% solution cleared the 6μ emulsion in about one minute while the 1% solution required 90 minutes to clear it. Both extremes damaged the blood cells considerably. A 10% solution cleared the plate in approximately 3 minutes, leaving the cells in a satisfactory condition for study.

Although it is the usual photographic procedure to fix for twice the length of time required for clearing of the emulsion, we feel that it is better to fix only until clear and then to wash for 20 to 30 minutes. Prolonged washing partially completes removal of the silver bromide left after the hypo treatment without damaging the cells.

Note added in proof: It has been found in this and other laboratories that the effective sensitivity of the NTB emulsion can be increased by keeping the relative humidity of the air in which it is stored at about 20% to prevent the fading of the latent image on the silver halide grain. We have used anhydrous calcium chloride, silica gel and various concentrations of sulfuric acid. The solid desiccants are more convenient. In some cases, the emulsion will become separated from the glass plate if the moisture content is reduced to approximately zero. Additional studies on latent image fading are underway.

[4]Data obtained in this laboratory and to be published soon.

THE USE OF PROTEIN SILVER FOR STAINING PROTOZOA

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ABSTRACT.—**When commercially prepared silver products suitable for staining protozoa by the Bodian silver technic apparently became unavailable, a substitute for Protargol was prepared as follows: 0.9 g. of gelatin is dissolved by heat in 100 ml. of distilled water; to this 0.1 g. of silver nitrate is added at 60°C.; this solution is poured into Columbia staining dishes (10 ml.) in which one or two drops of M/10 sodium hydroxide have been added. Copper is not used in the impregnating bath. Smears fixed in Hollande's or Schaudinn's fixatives are bleached and impregnated for 36 hours or more at 35°C. Impregnated smears are reduced with a mixture of hydroquinone and sodium sulfite, and toned with gold chloride as recommended by Kirby (1945).**

INTRODUCTION

The modification of the Bodian silver technic suggested by Kirby (1945) and Honigberg (1947) for the study of protozoa is especially useful for demonstrating the extranuclear structures of flagellates. This method requires the use of Protargol, a strong protein silver, sold by Winthrop-Stearns, Inc. In the past our most satisfactorily stained preparations were obtained by using strong protein silver bearing the control number 346BT. Recently, however, only Protargol-S (control numbers N170J and 335CB) has been available. After failing to obtain satisfactory results with these products, experiments were undertaken to produce a substitute protein silver suitable for staining.

According to the *U. S. A. Dispensatory*, 24th Edition, strong protein silver contains between 7.5 and 8.5% silver. It is prepared by reacting a silver compound with gelatin under alkalin conditions. With this procedure a protein silver solution has been prepared which has staining properties comparable to the most satisfactory Protargol.

In order to evaluate the usefulness of the protein silver solutions, smears of intestinal flagellates from the ceca of hamsters were used as test material. The method most successful with material from the hamster was then applied to intestinal protozoa from rat, mouse, frog, termite and culture.

Fixation

Kirby (1945) recommends Holland's cupric picro-formol mixture as a fixative preparatory to impregnation with Protargol. Schaudinn's fluid with 5% acetic acid added may also be used, although the anterior flagella of certain organisms stain less intensely than with Hollande's mixture. However, the cytosome appears more transparent when fixed in Schaudinn's fluid.

Bleaching

For more uniform results, it is advisable to bleach smears before impregnation, as recommended by Honigberg (1947).

Impregnation

The impregnation bath is prepared as follows: 0.9 g. of gelatin is dissolved by heat in 100 ml. of distilled water; to this 0.1 g. of silver nitrate is added at 60°C.; this solution is poured into Columbia staining dishes for cover slips[1] (10 ml.) in which one or two drops of M/10 sodium hydroxide has been added. The impregnation bath may be used more than once.

Gelatin. Although the physical and chemical properties of gelatins vary according to their source and method of preparation, those suitable for use in silver impregnation are readily obtainable. Granular gelatin, U. S. P., purchased from Eimer and Amend, and Knox gelatin of the same grade give similar results. Purified calfskin gelatin available from Eastman Kodak Co. is sold in sheet form. It is less convenient to handle and is useful only with preparations fixed in Schaudinn's fluid. The following gelatins were found to be unsatisfactory: Purified pigskin gelatin, Eastman Kodak Co.; Gold Label gelatin, A. H. Thomas Co.

Chatton and Lwoff (1936) used gelatin in greater strength than here recommended. An increase in the concentration of gelatin up to 10% has not been found advantageous.

Silver. Silver in the form of silver nitrate, reagent grade, has been used. Although various concentrations of silver nitrate from 0.05 to 1.0% were tried, optimum staining was achieved with 0.1 g. of silver nitrate per 100 ml. of gelatin solution.

Temperature. The temperature at which silver nitrate is added to the gelatin solution must be regulated to ensure successful impregnation. At 60°C. and above, satisfactory impregnation results; below 50°C. the impregnation bath is less effective.

[1]See Bowen (1929).

Time. Efforts to decrease the time of impregnation to an hour or less by immersing the smears in the impregnation bath maintained at 50°, 60°, and 70°C. resulted in workable but inferior preparations. Increasing the silver and gelatin concentration was not effective for shortening the impregnation period. The most satisfactory preparations were found in smears impregnated for 35 or more hours at 35°C.

Hydrogen ion concentration. At the time that these experiments were undertaken, it was hoped that the control of the hydrogen ion concentration might produce more uniform staining results. The use of three buffers (potassium acid pthalate and sodium hydroxide, potassium dihydrogen phosphate and sodium hydroxide, and sodium monohydrogen phosphate and potassium dihydrogen phosphate) indicate that ions other than hydroxyl and hydrogen ions must affect silver impregnation, for at identical pH-values there are distinct differences in the staining effects with the differently buffered protein silver solutions. Fairly acceptable stains were obtained only with protein silver buffered with potassium acid pthalate and sodium hydroxide at pH 6.4. At a higher pH much of the smear is lost in processing.

"*Activation*". According to Bodian (1936) success with Protargol depends upon the use of metallic copper, mercury or hydrogen ion in the impregnation bath. Copper, in varying amounts, interferes with impregnation when added to the protein silver solution prepared as suggested above.

Chatton and Lwoff (1936) reported that gelatin must be dissolved in saline solution for desirable silver stain. Sodium chloride introduced in this protein silver solution produced unsatisfactory results.

Modifications. With hamster material it is possible to obtain favorable slides without the use of sodium hydroxide in the impregnating bath. The addition of one or two drops of M/10 sodium hydroxide to 10 ml. of protein silver solution, however, improves the staining. With Knox's gelatin one or two drops of M/10 nitric acid yields more desirable effects with termite material. The use of nitric acid or the reduction of the amount of M/10 sodium hydroxide used is recommended where the stained organisms have an objectionable reddish tinge. Potassium hydroxide may be substituted for sodium hydroxide.

Precaution. The impregnating medium should not be exposed to intense daylight at any time.

Procedure

The following procedure is suggested:

1. Fix smears in the recommended fixative.
2. Wash in 50% alcohol to remove fixative.
3. Harden smears in 70% alcohol in which they may be stored.
4. Hydrate smears.
5. Bleach:
   a. Treat preparations in 0.5% potassium permanganate for two minutes.
   b. Wash in at least two changes of distilled water, one minute in each.
   c. Treat with 5% oxalic acid for two minutes.
   d. Wash in three changes of distilled water.
6. Impregnate smears in protein silver solution for 36 hours or more at approximately 35°C.
7. Reduction of silver. Rinse impregnated smears in distilled water and treat for 5 minutes in a reducing bath prepared by dissolving 1 g. of hydroquinone and 5 g. of sodium sulfite in 100 ml. of distilled water.
8. Wash in distilled water for one minute.
9. Toning. Immerse preparations for 4 minutes in 0.2% (1% may be used) yellow gold chloride. Staining is affected by the duration of the gold toning.
10. Rinse in distilled water.
11. Treat with 2% oxalic acid for three minutes. Variation of time in oxalic acid produces staining differences.
12. Wash for one minute in each of three changes of distilled water.
13. Treat with 5% sodium thiosulfate for 7 to 8 minutes.
14. Wash in running tap water for ½ hour or more.
15. Dehydrate and mount.

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A MODIFICATION OF THE SCHMUCK-METZ WHOLE-MOUNT TECHNIC FOR CHROMOSOME STUDY

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INTRODUCTION

Chromosome study in small organisms has been discouraged by the difficulties involved in embedding and sectioning and in analysis of serial sections, as pointed out by Schmuck and Metz in 1928. These authors outlined a technic for making whole mounts of Sciara eggs and suggested that it might be applicable to other small objects. The author has modified this technic somewhat in accordance with recent suggestions of other investigators and has used it with success on Arbacia eggs, Chaetopterus eggs and larvae, small nematods, small insect eggs and embryos, and insect ovaries.[1] In all cases exceptionally good fixation and staining of chromatin were obtained. For example, in Chaetopterus larvae with a large number of cells, chromosome studies can be made of all cells in the process of division. Literally, hundreds of specimens can be fixed, stained, and ready for mounting with three hours of work, and large numbers can be mounted in a short time.

METHOD

Material is fixed in shell vials. If it settles readily, as is the case with marine eggs and larvae and small nematods, fluids are poured over it and subsequently removed with a pipette. Objects which tend to float, such as insect eggs, are wrapped in lens paper sacs. These are transferred from one fluid to another and are not opened until material is ready for mounting. Kahle's fixative (distilled water 30 ml., 95% alcohol 15 ml., formalin 6 ml., glacial acetic acid 1 ml.) is used and is excellent for invertebrate material in general. Fixation of insect eggs is improved if they are punctured. This is unnecessary for other objects studied. The stain is made by bubbling sulphur dioxide gas into 0.5% basic fuchsin solution until it is decolorized. The sulphurous acid bath is prepared by saturating distilled water with sulphur dioxide gas (Rafalko, 1946). Shell vials containing specimens in normal HCl are placed in a water bath at

[1]This study was carried on with the aid of a research grant from the National Cancer Institute of the National Institute of Health, U. S. Public Health Service.

60°C. Transfer from water and xylene is made through triethyl phosphate (Nelsen, 1945). The regulation ethyl alcohol method can be used, but it tends to reduce intensity of staining. In mounting, a ring of rather thick balsam in xylene is placed on the slide, a drop of xylene with concentration of specimens is placed in the center of this ring, and a cover added. The schedule used is as follows:

1. Fixation, Kahle, ½ to 24 hours.
2. Distilled water, 2 changes, 1 hour each.
3. Normal HCl at room temperature, 10 minutes.
4. Normal HCl at 60°C., 10 minutes.
5. Leuco basic fuchsin, 2 hours.
6. Sulphurous acid bath, shortest time practicable.
7. Distilled water, 2 changes, 10 minutes each.
8. Triethyl phosphate, 2 changes, 15 minutes each.
9. Equal volumes of triethyl phosphate and xylene, 15 minutes.
10. Xylene.
11. Mount in balsam.

Discussion

All steps which proved to have little, if any, effect on the stain have been omitted. These include rinsing with water and sulphurous acid between HCl and fuchsin as well as changes of HCl and of sulphurous acid bath. Each investigator may, however, prefer to use his own schedule in these respects. In steps where very brief exposures to reagents are indicated in usual schedules for the Feulgen technic, it proved necessary, in this whole-mount method, to wait until specimens settled before changing fluids. This increase in time does not appear to affect results.

The author is convinced that many investigators are still wasting time and energy embedding and sectioning material which would be as good or better if prepared by the simple whole-mount method.

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A NEW TYPE OF TISSUE WASHER FOR MICROTECHNIC

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The process of preparing tissues for washing by means of the conventional gauze covered glass tubes is tedious, frequently cumbersome, and the results obtained by the usual methods of washing often leave much to be desired. If the tubes are placed upright in a Buchner funnel, the adsorption of air bubbles and the shifting of the tubes result in a slow, uncertain washing which is largely by a slow diffusion as little water actually passes through the tubes containing the tissues. The washing bobber of Gavins[1] is an improvement in that the bobbers are easily and quickly loaded and can be used indefinitely, but the washing is still done by a slow diffusion. To wash tissues thoroughly and in the minimum time the water should either flow over the tissues or be changed frequently. Devices for washing in running water have been designed by a number of investigators[2] which meet the above objections, save that they are complicated in construction and require considerable time in loading and unloading the tissue containers.

The device herein described (Fig. 1) has the advantages of simplicity in construction, loading and unloading, and it washes quickly and thoroughly. The device is fully automatic; the water being completely changed at wanted intervals.

The apparatus, as illustrated, consists of a washing column about 18 inches high and 1½ inches in diameter made by cutting off two ends of a fluorescent light tube. The bottom of the tube is closed with a rubber stopper which is bored to receive two short lengths of glass tubing of about ¼ and $\frac{7}{16}$ of an inch in diameter respectively. To the glass tubes are attached rubber or plastic hoses, the smaller one leading to the faucet for filling the tube, the larger leading to the sink for emptying. The dimensions given above may be changed as

[1]Gavins, F. W. 1942. The washing bobber. Stain Techn., **17**, 131.

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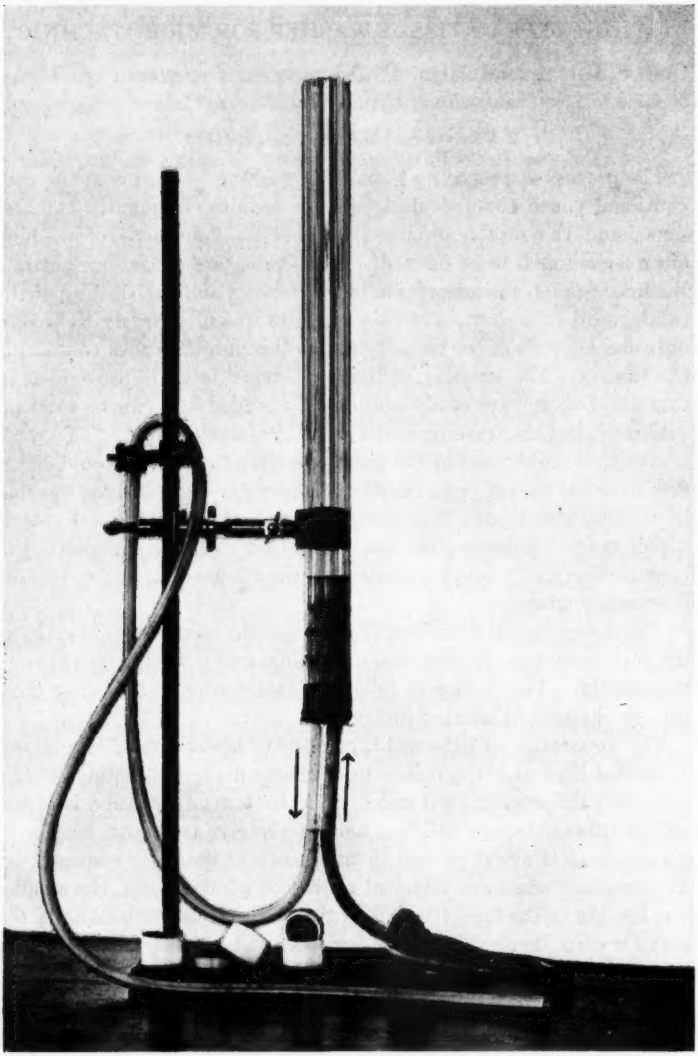

FIG. 1.—Tissue washer assembled. Three washing cylinders are enclosed in position in the washing column. Three other washing cylinders are lying on the base of the ring stand to show the parts. The arrow pointing upward is the intake hose and the downward pointing arrow indicates the exhaust.

desired save that it is essential for proper functioning that the exit tube be as large or larger than that given.

The washing column is held in a vertical position by a ring stand. By looping the exhaust hose (indicated in Fig. 1 by arrow pointing downward) at any desired height, the column can be filled to the corresponding height and then a siphon is started which rapidly and completely empties the column. In a matter of seconds (depending on rate of water inflow) the tissues are again covered and there is no danger of them drying out.

For tissue containers which are quickly loaded or unloaded and can be used permanently, we cut a 1¼ inch tube of polyethylene tubing into 1 inch lengths. To one end of the tube is cemented a trimmed piece of fine mesh copper screen. The cementing is accomplished by heating the screen and gently pushing the polyethylene tube into it. Hardening occurs quickly and the seal is secure. To make a cap for the washing cylinder, a section of the same kind of tubing was cut to a length of ½ inch. From the side of this a small piece was removed by cutting and the cylinder rejoined by gentle heating and pressure. To this cap was affixed a copper screen and two parts of the cylinder joined, the smaller one fitting snugly within the larger unit. At least a dozen of these cylinders containing tissues can be washed at one loading of the washing column.

We experienced some difficulty, when the apparatus was new, in wetting the copper screen and the cylinders. They would hold air bubbles by adsorption and float with the ascending water. Several drops of a wetting agent such as Morpholine or Turgitol poured down the washing column reduced the surface tension and removed the bubbles. However, until the system has been used repeatedly, it would be safer to drop a weight (such as a short piece of metal pipe attached to a string for removal) down the washing column on top of the washing cylinders. Tissues such as root tips fixed with salts of heavy metals for chromosomal or cytoplasmic studies are uniformly washed in four hours.

A COMPARISON OF DEFORMALINIZING TECHNICS

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ABSTRACT.—**Five methods of removing bound formalin from tissues were studied with the Cajal, Del Rio-Hortega, and Feulgen technics used as indicators of efficiency of removal. The tissues had been fixed in 10% formalin for varying periods from one week to several years. The most effective means of eliminating the effects of formalin on staining was two 24-hour changes of 20% aqueous chloral hydrate. Tissue so treated (in the block) gave good results with both the Feulgen and Cajal technics. The Del Rio-Hortega silver carbonate method did not differentiate microglia and oligodendroglia, although astrocytes, unstained in untreated material, stained well after deformalinizing.**

Tissues fixed in 10% formalin for a week or more are unsuited to certain types of special staining, such as Cajal's silver stain for nerve fibers and Del Rio-Hortega's method for neuroglia. Moreover, tissues so fixed are quite unsuited to the Feulgen reaction (Feulgen and Rossenbeck, 1924) because of poor nuclear differentiation after staining. Methods for restoring the desired stainability by removing or replacing the formalin have been devised, but no critical study of their relative effectiveness seems to have been made.

We have compared the staining results obtained after five types of procedures for formalin removal. Schedule 1 gives the details of the procedures. An objective means of evaluating the merits of these procedures was provided by comparing the results of Feulgen stains and silver methods (Schedule 2).

Schedule 1. Treatment of formalin-fixed tissues to remove bound formalin.

A. BLOCKS

1. Washed 1–8 days by running tap water followed by 2 changes of distilled water of 6 hours each (suggested by Bauer, 1932).

[1]Publication Number 522.

[2]On leave of absence as Rockefeller Research Fellow in Neurology, Dept. of Anatomy, Northwestern University Medical School.

2. Two changes of 24 hours each in various mixtures of distilled water, alcohol, and ammonia. Two volumes concentrated ammonia water, 3 of distilled water, 5 of absolute alcohol, seemed the most effective (based on a method suggested by Cajal, 1910).
3. Two changes, 24 hours each, of 2% chloral hydrate in 50% alcohol (method of Cajal, 1910).
4. Same as 3, except 20% chloral hydrate in water (Vaz Ferreira modification of Cajal's method).

B. Sections

1. Arcadi's method (1948): 12–30μ frozen sections placed in strong ammonia water and washed for 24 hours with a slow stream of tap water coming from a 1-mm. nozzle. The washing is repeated once.

Schedule 2. Silver and Feulgen stains used to test the efficiency of treatments given in schedule 1.

A. The Del Rio-Hortega silver carbonate method as given by Conn and Darrow (1947).

B. A modification of Cajal's silver nitrate technic as follows:

1. After completion of the deformalinizing, blocks of tissues were treated overnight in ammoniated alcohol (95% alcohol, 100 ml.; strong ammonia water 3–5 drops).
2. They were washed for 15 minutes in distilled water and placed in aqueous 2% silver nitrate solution for 16–24 hours.
3. The silver nitrate solution was renewed and the blocks allowed to continue staining for 14–25 days.
4. On completion of staining, the blocks were washed for one minute in distilled water and then reduced overnight in 5% aqueous pyrogallol solution.
5. After reduction, the blocks were washed for fifteen minutes, dehydrated through graded alcohols to xylene, embedded in paraffin, sectioned, mounted, cleared, and covered.

C. Both the standard (slide) Feulgen technic (de Tomasi, 1936) and the block method (Lhotka and Davenport, 1947, 1949) were used for the tests.

Table 1 shows the graded results with each of the three staining methods with each of the five deformalinizing technics and untreated control specimen.

The tissues used in the experiment consisted of spleen, intestine, lung, salivary gland, and nervous tissue of rats, and also nervous tissue of cats. The rat tissue was fixed in 10% formalin for varying periods up to three months. The cat tissue had been stored in 10% formalin for a period of about three years.

Washing of tissues (Bauer, 1932) with running tap water, even though prolonged for 8 days, was an inadequate means for removing bound formalin. The best results after plain washing were obtained

with the Feulgen slide technic, in which fair nuclear differentiation was obtained. The results, however, were inferior to those obtained with similar tissues when formalin had not been used in fixation. Likewise, plain washing did not improve subsequent silver staining.

The Arcadi method (1948) gave staining results that were superior to those obtained by plain washing, but it did not seem to remove formalin as effectively as the procedures given below.

The alcohol-ammonia and the alcoholic chloral hydrate methods, both based on suggestions of Cajal (1910), gave satisfactory results with the Feulgen reaction. Good differential staining was obtained with both methods. They were, however, unsatisfactory for silver technics.

TABLE 1. THE RELATIVE EFFECTIVENESS OF METHODS USED TO DEFORMALINIZE TISSUE, BASED ON SUBSEQUENT STAINING.

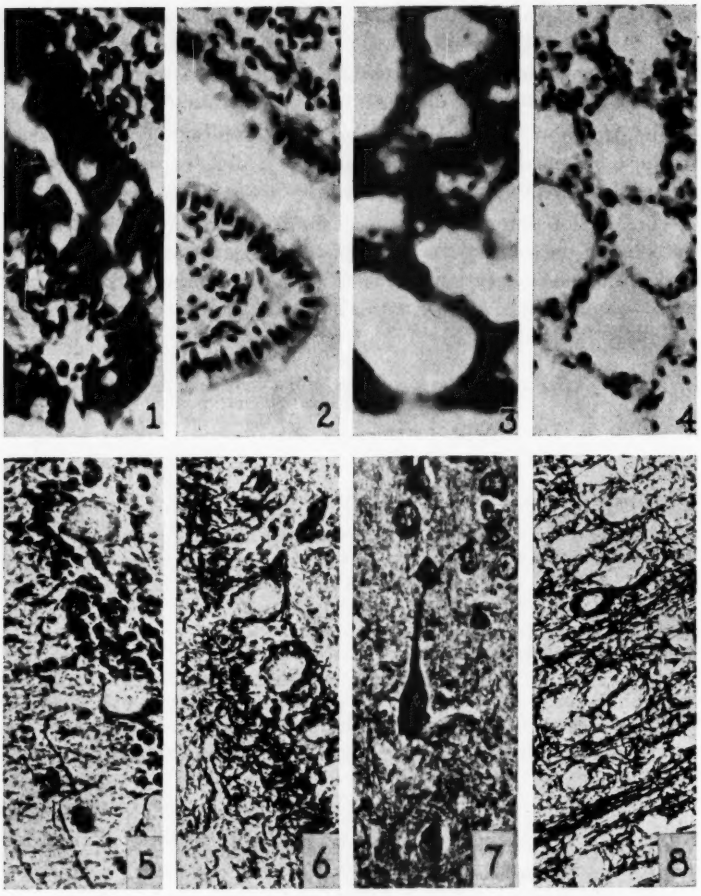
No effect – to greatest effect ++++

Deformalinizing Technic	A. Del Rio-Hortega Silver Carbonate Technic	B. Cajal Silver Nitrate Technic	C. Feulgen Stain Slide Block	
Untreated Control	–	–	–	–
1. Prolonged Washing	–	–	++	–
2. Alcohol-Ammonia	–	++	+++	+++
3. Alcoholic Chloral Hydrate	+	++	+++	+++
4. Aqueous Chloral Hydrate	++	++++	++++	++++
5. Arcadi Strong Ammonia	+	++	+	

The aqueous 20% chloral hydrate treatment was the most effective of the five methods. Satisfactory results were obtained with both the Feulgen and Cajal stains.

The rationale by which a strong chloral hydrate solution takes out formalin is not entirely clear. Although the chemical relationship between trichloracetaldehyde and formalin appears to be close, when trichloracetaldehyde is hydrated to form chloral hydrate, its structural form is so altered that it no longer behaves as a true aldehyde. Chloral hydrate is Schiff (Feulgen) negative, and this is a test that is considered specific for the aldehyde radical. The chloral hydrate, either by replacement or by elution of bound formalin apparently alters the tissue so that it no longer exhibits staining effects attributable to formalin.

Comparisons between similar tissues showing the effects of the 20% chloral hydrate deformalinizing technic are shown in Fig. 1 to 8. The Feulgen sections, Fig. 1–4, following chloral hydrate treatment, show strong selective nucleal reactions in contrast to the diffuse staining of the untreated controls. The Cajal technic, Fig. 5–8, clearly



Explanation of Fig. 1–8. Section thickness: 6μ. Magnification: 1–4, ×125; 5–8, ×150. Sections 1–4, rat, three months in 10% formalin; 5–8, cat, approximately three years in 10% formalin. The odd numbers show sections from blocks of untreated material. The even numbers are sections from blocks which were deformalinized by 20% aqueous chloral hydrate. Each pair of sections represents material from a different animal, and the paired sections are within 1 mm. of each other. Sections 1–4 were stained by means of the Picro-Feulgen block method while sections 5-8 were stained by the Cajal silver nitrate technic.

Fig. 1–4 illustrate the contrast between diffuse staining of untreated intestine and lung as compared to good differentiation shown by deformalinized material as demonstrated by the Picro-Feulgen block stain.

Fig. 5–8 demonstrate the superior staining of neural elements, especially fibers, in deformalinized cerebellum (6), and cortex (8) with the Cajal silver nitrate technic.

demonstrates the neural elements with a minimum of precipitation in the treated sections in spite of over two years storage in formalin.

The results with the Del Rio-Hortega silver carbonate method could not be considered satisfactory as a means of staining microglia and oligodendroglia in our material. Differentiation of these cells was not obtained, although astrocytes often stained well after formalin removal by 20% aqueous chloral hydrate.

When silver stains are made after deformalinization, it is generally necessary to lengthen the time of impregnation. It was necessary to leave tissue blocks for the Cajal stain in the silver solution for at least two weeks, and at least twenty-four hours for the Del Rio-Hortega method.

The recommended technic is as follows:

1. Wash blocks of formalin-fixed tissue, not over 5 mm. in thickness, for 15 minutes in distilled water.
2. Transfer to 20% aqueous chloral hydrate solution for 24 hours. Change the solution and let the block remain in it for an additional 24 hours.
3. After removal from the second change of chloral hydrate wash for 15 minutes in distilled water. The tissues are now ready for whatever subsequent procedure is desired.

It must be remembered that deformalinizing is only a substitute for regular methods of fixation adapted to specific staining technics. Results may not be as good as those that would have been obtained if the proper fixative were used originally, but a greater variety of stains can be secured.

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A MODIFICATION OF MAYER'S MUCIHEMATEIN TECHNIC

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ABSTRACT.—**A relatively simple technic giving consistent results has been evolved from Mayer's mucihematein technic[1] by substituting hematoxylin for hematein and omitting the nitric acid. The hematoxylin is oxidized with sodium iodate ($NaIO_3$).**

This modification is effective on the same types of mucin as Mayer's original mucihematein. With this modified technic, mucin stains a deep violet, cell nuclei pale gray blue, and connective tissue pale gray to colorless in tissues fixed in all the more common fixatives. The modified stain retains this selectivity for at least 200 days.

In the course of recent mucin studies, it was found that Mayer's mucihematein stain lost its selectivity after one or two days. By omitting the nitric acid the usable period was prolonged up to 3 months, after which the mucin stained too pale and with such intense staining of nuclei and connective tissue that tinctorial differentiation was difficult.

Since hematein is an oxidized hematoxylin, trials were made using hematoxylin oxidized with varying amounts of sodium iodate, from 0.01 g. to 1 g. sodium iodate per g. of hematoxylin. In addition, one solution was allowed to ripen spontaneously by omitting sodium iodate altogether. Two series were run; one with and one without nitric acid. Tests were made daily for 22 days, and thereafter at 30, 60, 90, 120 and 200 days. In the series without nitric acid all of the 0.04–0.4 g. sodium iodate solutions were effective when used fresh, and the 0.04–0.1 g. sodium iodate solutions remained effective for at least 200 days. The 0.2–0.4 g. solutions showed variability in nuclear and connective tissue staining after 3 months. Those solutions with less than 0.04 g. sodium iodate did not stain unless allowed to ripen for 3 to 4 days. However, they retained their selectivity for at least 200 days. The solution without sodium iodate required 4 days to ripen and gave satisfactory results up to 3 months, thereafter exhibiting variabilities in nuclear and connective tissue staining. The solution of 0.5 g. sodium iodate did not stain until 4 days after

[1]Lillie, R. D. 1948. Histopathologic Technic. Blakiston, Philadelphia.

preparation. At that time, the mucin stained a deep violet but it was too inconsistent thereafter to be of use. The 0.6 g. sodium iodate solution did not stain mucin until 7 days after preparation. After 11 days this solution still stained the mucin but with considerable variability in the staining of nuclear and connective tissue. The 0.8 and 1.0 g. sodium iodate solutions were never satisfactory.

The series with nitric acid were not satisfactory; the 0.04–0.06 g. sodium iodate solutions were the only ones to stain when fresh but they became ineffective after 3 days.

The Technic

Preparation of stain:

Hematoxylin C. I. No. 1246		1 g.
70% ethyl alcohol		100 ml.
Aluminum chloride		0.5 g.
40 to 100 mg. sodium iodate (4 to 10 ml. of 1% aqueous sodium iodate)		
70% ethyl alcohol	qs.	500 ml.

Dissolve the hematoxylin in 100 ml. 70% ethyl alcohol, add the aluminum chloride and sodium iodate and make up to 500 ml. with 70% ethyl alcohol.

Procedure:

1. Deparaffinize sections and bring to water as usual.
2. Place on staining rack and rinse several times with *distilled water.*
3. Place 2 ml. of modified mucihematein stain on each slide and allow to remain 5 to 10 minutes.
4. Drain off stain and wash with *distilled water* 3 times for 5 minutes each.
5. Dehydrate in 2 changes of 95% alcohol, 2 changes of absolute alcohol, absolute alcohol and xylene (50:50), and 2 changes of xylene.
6. Mount in polystyrene, clarite, permount, or gum damar, etc.

Discussion

Hematoxylin, more readily obtainable and less expensive than hematein, gives more consistent results and is a reliable substitute for hematein in the Mayer mucihematein technic. By spontaneous oxidation or by oxidation with sodium iodate and omitting the nitric acid, the staining solution remains effective for at least 200 days.

I am indebted to Dr. R. D. Lillie for suggestions and advice.

STAINING PINEAL PARENCHYMA BY A MODIFIED HORTEGA METHOD AFTER PARAFFIN EMBEDDING

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ABSTRACT.—**Fresh pineal glands are fixed in 10% formalin at room temperature for about 3 days. After washing, dehydrating and clearing they are embedded in paraffin, sectioned and mounted. The tissues are placed in 10% silver nitrate for 24 hours, washed and impregnated in strong silver carbonate. The sections are reduced in 10% formalin, washed and toned in gold chloride, fixed in 5% hyposulfite, counterstained with erythrosin and mounted in Canada balsam. The processes of the pineal parenchymatous cells of the sheep, cow and man have been successfully stained by this method.**

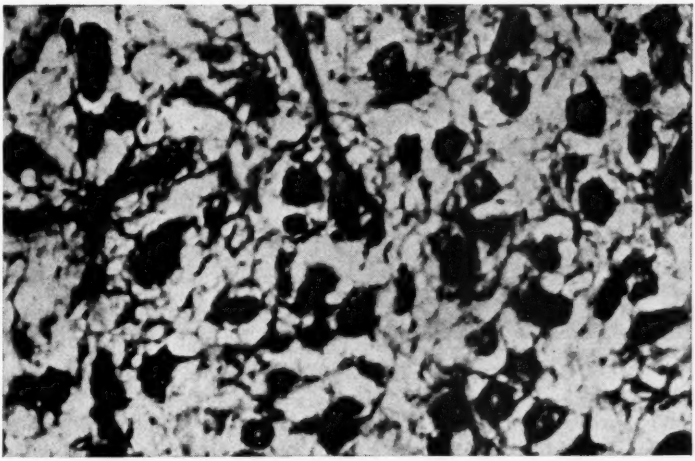

FIG. 1.—Photomicrograph of pineal parenchymatous cells of sheep. ×800.

Although the pineal is one of the smallest organs in the body, its phylogeny, embryology, histology and function are poorly understood. Gladstone and Wakeley (1940, p. 397), describe in the human pineal the following elements, namely: (1) astrocytes, (2) parenchyma or pineal cells, (3) mesodermal elements (a) fibrous capsule and trabeculae, (b) microglia, (4) cells and fibers which resemble nerve

cells and nerve fibers, (5) blood vessels. The bulk of the pineal consists of the parenchyma or pineal cells. Their nuclei and part of their cytoplasm had been previously described but their processes were first stained by Del Rio Hortega (1922) by a modification of his silver carbonate method. Over the intervening years many papers have been published on the histogenesis and histology of the pineal and the histology of pinealomas; however, very few of these investigators have successfully stained the processes of the parenchymatous cells.

Beginning in 1937 we made a series of studies on the histogenesis and histology of the neurohypophysis. All of our earlier material was prepared by using frozen sections and staining by the Hortega

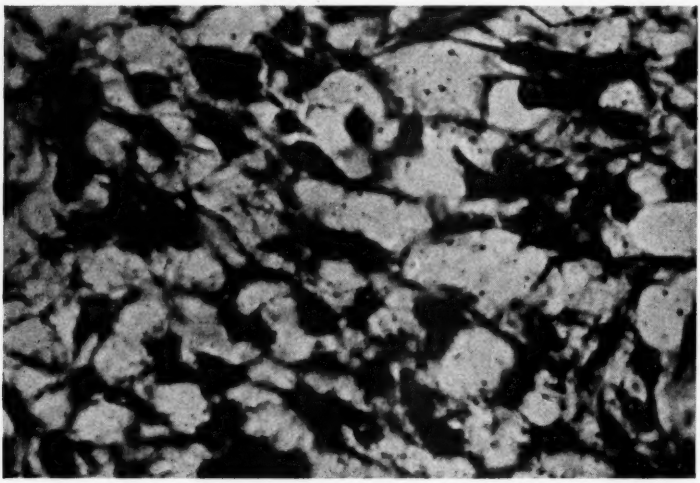

FIG. 2.—Photomicrograph of pineal parenchymatous cells of cow. ×800.

silver carbonate method. The advantages of using silver carbonate for staining after paraffin embedding and sectioning were described by Shanklin (1943).

A modification of the Hortega silver carbonate method for staining the processes of the pineal parenchymatous cells, after paraffin embedding and sectioning is as follows:

1. Fix the gland as soon as it is removed in 10% formalin (Merck blue label 40%) for three days at room temperature of about 24°C.
2. Wash for two hours in distilled water to which 6 drops of ammonium hydroxide are added for each 100 ml. of water.
3. Wash again in distilled water for a few minutes and dehydrate

the gland in 70%, 80%, 95% and absolute alcohol, leaving for three hours in each.

4. Clear in cedar oil over night, followed by pure xylene for half an hour.

5. Infiltrate with paraffin (melting point 48°C.) for three hours.

6. Embed in pure paraffin.

7. Cut sections 7–10 μ, fix to slides by the albumen-water method and dry thoroughly.

8. Heat the slide gently to soften the paraffin and pass through two changes of xylene.

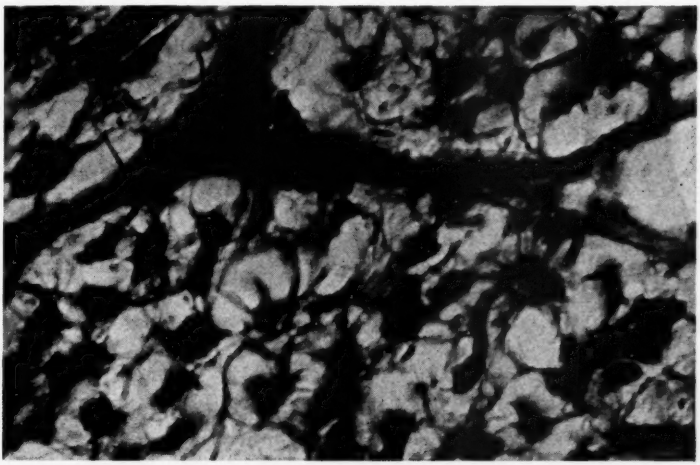

FIG. 3.—Photomicrograph of pineal parenchymatous cells of man. ×800.

9. Pass slides through absolute alcohol, 95% and 80% alcohol for one to two minutes in each.

10. Wash in three changes of distilled water for three minutes each.

11. Place slides in 10% silver nitrate to which three drops of pyridine for each 10 ml. are added and keep in the dark at room temperature 24°C. for 24 hours.

12. Wash in distilled water and sensitize by placing in 5% sodium sulfite for one hour. This step may be omitted, but sometimes this is necessary as it acts as a rejuvenator.

13. Wash in distilled water to which 2 drops of pyridine for each 10 ml. are added for 1 to 2 minutes followed by two changes of distilled water.

14. Impregnate in strong silver carbonate at room temperature for 2 to 5 minutes.

Formula:

Silver nitrate (pure crystals), 10% solution	100 ml.
Sodium carbonate, pure, 5% solution	300 ml.
Ammonium hydroxide, just sufficient to dissolve the precipitate	
Distilled water	100 ml.

15. Dip in distilled water for a few seconds, without agitating.

16. Reduce in 10% formalin (Merck blue label), to which 4 drops of pyridine are added for each 10 ml., for one minute agitating gently.

17. Wash in distilled water.

18. Tone in yellow gold chloride (1 g. to 500 ml. distilled water) for a few seconds to one minute. Time should be checked carefully.

19. Fix in 5% hypo for 1 to 2 minutes.

20. Wash and counterstain lightly with 1% erythrosin or safranin.

21. Dehydrate in 80%, 95% and absolute alcohol.

22. Clear in two changes of xylene, mount in neutral Canada balsam and cover with cover slips.

> Purity and good quality of formalin, silver nitrate, and sodium carbonate are essential for successful results.

The parenchymatous pineal cells have been successfully stained by the Hortega silver carbonate method after paraffin in the human, sheep and cow.

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MODIFIED METHODS FOR STAINING THE PRIMORDIAL CELLS OF GOSSYPIUM HAIRS[1]

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Outer epidermal cells of the ovules of the genus *Gossypium* contain fat globules in the cell sap at an early stage of development. These globules disappear gradually as the initiation and development of the hair takes place. Observers dealing with the epidermal cells of the cotton ovules before anthesis, often experience difficulty in distinguishing the nuclei from the oily material due to the fact that the oil drops cover the nucleus and make it indistinguishable. The following modified technic has been found useful in a study (in press) to clarify the behavior of the nucleus of the epidermal cell before initiation of the *Gossypium* seed hairs. Of many fat solvents which were tried, petroleum ether was found to have the least effect on the shape and structure of the cell.

The Method (Using Crystal Violet Iodine)

Slides of paraffin sections are left in xylene for 15 minutes to remove as much of the paraffin as possible. It is possible to leave the sections longer in the xylene, but it is not recommended, because of the change in the structure of the cells. Sections are then placed in a jar of petroleum ether (another fat solvent), covered tightly to prevent evaporation. The effect of petroleum ether, in penetrating the cell walls and dissolving the oil droplets, make its use preferable to leaving the sections in xylene for a longer time. Xylene is miscible with petroleum ether and so the sections will remain clear and distinct. Of the many solvents used, petroleum ether was found to be the fastest in dissolving the fat-drops inside the cell without affecting the general structure or the shape of the cells.

It is necessary to put the slides again in xylene and then to pass them as indicated to absolute alcohol for short intervals. This was found to be much better than putting the slides from xylene to absolute alcohol or passing them through the different alcohols. If the slides are left in absolute alcohol longer, the cells shrink. On the other hand, if they are passed to the different alcohols at shorter intervals, an emulsion will be formed between the remnants of the xylene in the section and the water in the diluted alcohol. Best results were obtained when following the steps presented.

Ovules of different species and varieties within species differ in oil

[1]This work was started at the Texas Agricultural Experiment Station, College Station, Texas, and continued at the University of Arizona, Plant Breeding Department. It was approved by the Experiment Station, for publication on February 28, 1949.

content; therefore, the time they should be left in the solvents depends upon the material and the thickness of the sections. Sections 8 μ in thickness are left for 15 minutes, which was found to be sufficient and had no effect on the cell structure. As the section increases in thickness, it requires a longer time in the solvents which will have some effect on the cell structure.

After removal of oil droplets, the chromatic material in the cell is the only material not completely destained by clove oil. In sections, treated as indicated in the present method, the nuclei as well as the nucleoli are clearly differentiated for study. (Results in press). The modification of the standard method, as shown above, is the general change from xylene to absolute alcohol, which keeps the cell in the same shape and size, clear from water-xylene emulsion.

Using the above technic, it is found possible to explain some of the phenomena that were observed before (cf. Heiba, Science, 1948).

The method in brief:—

Xylene	15 minutes
Petroleum ether	15 minutes
Xylene	10 minutes
Xylene ¾ + absolute alcohol ¼	5 minutes
Xylene ½ + absolute alcohol ½	5 minutes
Xylene ¼ + absolute alcohol ¾	5 minutes
Absolute alcohol	2 minutes
Alcohol, 95%	5 minutes
Alcohol, 70%	5 minutes
I-KI (1% potassium iodide, 1% iodine in 70% alcohol)	10 minutes
Water	5 minutes
Crystal violet, 1% in water	15 minutes
Water	5 minutes
I-KI	10 minutes
Water wash	2 seconds
Alcohol, 50%, + I-KI + picric acid	10 seconds
Alcohol, 95%	2 seconds
Alcohol, absolute	2 seconds
Xylene ⅔ + alcohol ⅓	2 seconds
Destain with clove oil	
Xylene	15 minutes
Mount in permount	

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APPLICATION OF FLUORESCENCE MICROSCOPY AND PHOTOMICROGRAPHY TO WOODY TISSUES

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ABSTRACT.—**A procedure is described for making preparations of woody tissues for visual observation or photography by incident-light fluorescence microscopy. The chief advantages of the technic are the following:**

(1) Reliable recognition of anatomical characteristics in wood without ordinary time-consuming histological technics.

(2) Examination of relatively larger surface areas of wood blocks than by usual methods.

(3) Visual observation and, if desired, photography of tissues and cell structure in dry or in nearly natural or fresh condition.

(4) Marked color contrast without the use of stains in many tissues, including specific types of cells comprising them.

(5) Improved color contrast by use of Congo red with aspects not usually obtained by other methods.

For comparative investigations of wood anatomy, it is often necessary to examine hundreds of specimens in order to determine significant differences with any degree of certainty. Methods that permit the quickest and most reliable recognition of the different structural and chemical characteristics are highly desirable for such studies. The usual histological and microchemical methods that require much time and complicated procedures should, if possible, be resorted to only to determine the finer structural details.

The procedure to be described was developed at the Forest Products Laboratory during the study of oak specimens by incident-light fluorescence microscopy on the smoothed surface of small blocks. This eliminated the cutting of sections and the attendant complicated histological procedures and allowed low-power (30–200 times magnification) examination of relatively large surface areas. It also permitted observations of tissues in nearly natural conditions. Photomicrographic records have been made, when desired, in black and white. Transparencies have been produced with daylight type 35 mm. color film, although long exposures were required because of

[1]Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

the comparatively low intensity of the fluorescent light. Specimen blocks were surfaced with a sliding-type microtome or were prepared freehand with a small microtome knife. Small blocks of fresh or preserved material were placed in a small glass dish (Fig. 1), oriented in loosely crumpled aluminum foil, and immersed in either distilled water or 70% alcohol. When photomicrographs were to be made, the air was always previously evacuated from the block with an aspirator. During this procedure the glass dish with specimen was

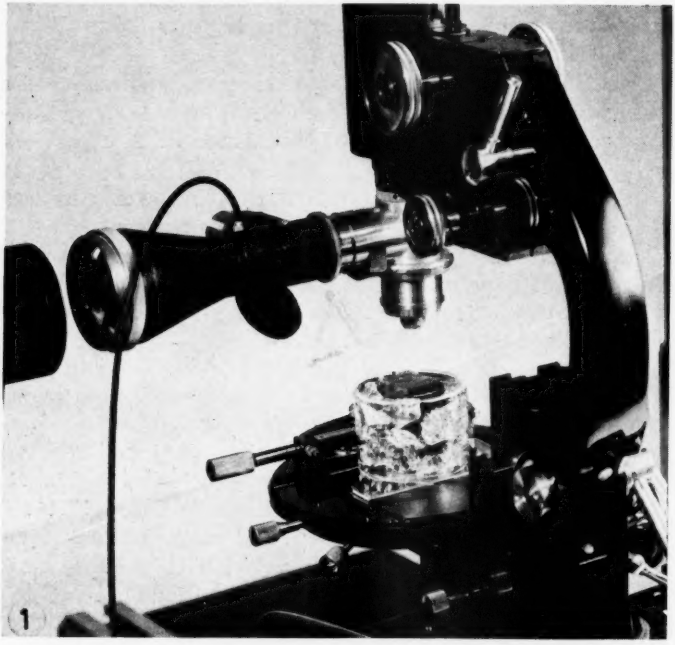

FIG. 1.—Detail of fluorescence microscope showing prepared wood block for surface examination or photomicrography. (M75567F)

submerged in water or in 70% alcohol in a beaker, was then placed in a low-pressure-vacuum container, and after evacuation of the air was removed from the chamber. The surface of the specimen was leveled to the sides of the dish by pressing with a glass microscope slide. The liquid was lowered with a pipette until just below the surface of the specimen, when a thin No. 1 or No. 0 cover glass was immediately placed over the surface in order to avoid bubbles and to prevent surface drying of the specimen.

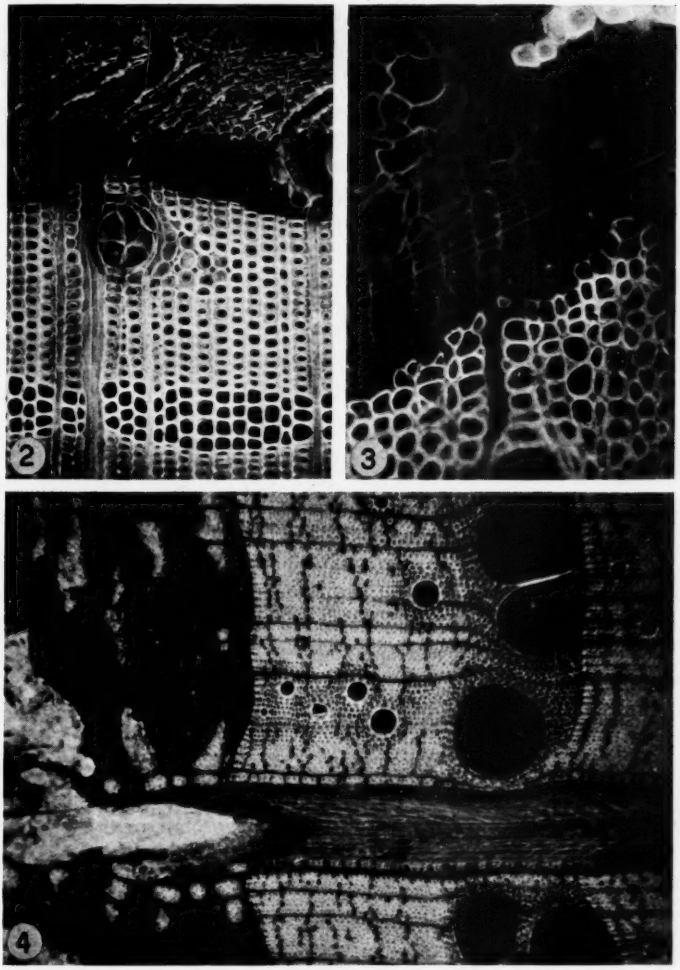

FIG. 2.—Fluorescence photomicrograph, transverse surface view of longleaf pine (*Pinus palustris* Miller) showing resin-canal tissue, summerwood and springwood tracheids, ray tissues and phloem tissues. Magnification approximately 75×; stain Congo red; achromatic objective 11× with quartz ring condenser; eyepiece 6×. (M82768F)

FIG. 3.—Fluorescence photomicrograph, transverse surface view of blackjack oak (*Quercus marilandica* Muenchh.) showing the July appearance of the cambial region with adjacent phloem and xylem tissues. Magnification approximately 220×; stain Congo red; achromatic objective 22× with quartz ring condenser; eyepiece 10×. (M82770F)

FIG. 4.—Fluorescence photomicrograph, transverse surface view of blackjack oak showing an annual ring and adjacent phloem tissues. Magnification approximately 40×; stain Congo red; achromatic objective 5× with ring condenser; eyepiece 6×. (M82769F)

A specialized microscope and a photomicrographic base constructed for the purpose was used for examination and photography of specimens. This equipment produced dark-field illumination with incident ultraviolet rays. The light source consisted of a direct-current carbon arc with radiation (wave lengths between approximately 3,200 and 4,000 Å) passed to the specimen through quartz or ultraviolet transmitting glass. Structural details of the tissues showed very clearly (Fig. 2, 3, and 4), including specific types of cells comprising them. Often there was marked color contrast without the use of fluorochromes or stains. Although preparations are temporary, photomicrographs and descriptions of selected areas can serve for permanent records. This technic has proved applicable for extensive and detailed study of oak and pine.

Block staining of oak and pine with Congo red[2] in a 1% aqueous solution produced improved color contrast. The time of staining was varied from 15 minutes to as much as 1 hour. The blocks were then washed directly in running tap water for a few minutes, and preparations were made as described above. In oak, the cambial region, the main phloem tissues, and most rays showed red, in contrast to the blue fluorescence of tracheids and phloem fibers. Resin ducts in pine showed excellent red color contrast to the blue of surrounding tracheids in a way not usually obtained by other methods.

In addition to its use for examination of woody tissues in preparations as described, fluorescence microscopy has proved valuable for study of dry preparations, such as penetration of paints and enamels, surfaces of papers, the dispersion of resins in various pulp mixtures, and for study of glue layers. Sectioned material, stained or unstained, and mounted in a very low fluorescing medium, also gives very good results. Fluorescence microscopy and photomicrography show great promise for remarkably revealing structure of woody and herbaceous tissues.

[2]Color Index No. 370, certified for use in histology by the Biological Stain Commission, Geneva, N. Y.

THE HYDROGEN-ION CONCENTRATION OF THE PRIMARY FIXING SOLUTIONS

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It is felt that basic to an understanding of the role of the hydrogen-ion concentration in fixation of tissues is a knowledge of the pH values of the chemicals which are commonly employed in making the numerous fixing mixtures. Petrunkevitch and Pickford[2] measured the pH of thirty-four fixatives and found all but one within the acid range. They did not, however, consider the primary solutions used in respect to their hydrogen-ion values.

Table 1. pH Values of Twenty-one Fixing Solutions

Readings taken in fresh condition and after standing for one month. All were made in 1% concentrations unless otherwise specified.

Fixing solutions	pH Fresh	pH After standing one month
Sulfuric acid	0.6	0.6
Nitric acid	0.9	0.9
Chromic acid	1.2	1.2
Trichloracetic acid	1.2	1.2
Picric acid (sat. aq.)	1.3	1.3
Formic acid	2.0	2.0
Acetic acid 5%	2.3	2.3
Platinum chloride	2.5	2.5
Mercuric chloride (6.9%)	3.0	3.0
Formaldehyde (Comm.)	3.4	3.4
Potassium bichromate 3%	3.9	4.2
Copper sulfate	4.4	4.4
Potassium chlorate	5.3	4.4
Potassium chromate	5.4	5.0
Sodium sulfate	5.8	5.4
Osmic acid	6.1	6.0
N-butyl alcohol—absol.	6.2 ? variable on reading	
N-propyl alcohol—absol.	6.7 ? variable on reading	
Acetone—absol.	7.0 ? variable on reading	
Chloroform—absol.	7.2 ? variable on reading	
Absolute alcohol (ethyl)	8.4	6.5

The included table gives the pH-values of 21 single fixing solutions in the concentrations usually utilized in making various well known

[1]Aided by a grant from the Committee on Scientific Investigation of the American Medical Association.

[2]Petrunkevitch, A., and Pickford, Grace E. 1936. On the relative acidity of histological fixing fluids. Anat. Rec., **65**, 461–6.

fixatives. They were measured with a Beckman pH meter, Model H-2, both in the fresh condition and after standing for one month. The listing is in the order of their decreasing acidity. As can be seen, they vary from a pH of 0.6 to 8.4 but 17 are on the acid side. Those having an unstable pH on standing are the following: potassium bichromate and chlorate, absolute alcohol, chloroform, *n*-butyl alcohol, *n*-propyl alcohol and acetone. The last four are extremely sensitive and show decided changes on the slightest agitation making it difficult to take accurate readings.

We have measured the pH of 32 more fixing mixtures than did Petrunkevitch and Pickford (1936) making a total of 66 recorded values. Collectively, 62 (94.0%) are within the acid range in respect to the hydrogen-ion concentration.

In conclusion, it would seem that the acidic nature of most all fixatives may be attributed to the characteristic pH values of the primary fixing solutions and possibly to certain laws of hydrogen-ion concentration which function when they are mixed together.

THE DRY PRESERVATION OF FIXED PLANT MATERIAL

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Received for publication July 14, 1949

ABSTRACT.—**A method for the dry-preservation of fixed plant material, root tips and buds, is described. The method seems to be advantageous on long expeditions and when material has to be sent away.**

The material is transferred from the fixative to 70% alcohol (3 changes, ½ hour in the last). It is dried on blotting-paper. The dried material may be preserved a long time. Material kept dry for 4½ years has proved to be quite satisfactory. Drying has been tried after fixation with CRAF-solutions (Webber and Randolph modifications) and fixatives containing osmic acid (Fleming-Benda and 2BD).

The dry material is swollen by keeping for 2 days in 10% alcohol. It is embedded in paraffin according to the usual method. A satisfactory staining has been obtained after these fixatives using iodine-gentian-violet and Feulgen stainings. In addition to chromosome counts dry material may be used for chromosome morphology studies.

Dried material fixed in aceto-alcohol (1:3) has not turned out to be specially suitable for squash preparations owing to the fragility of the chromosomes. If strong pressure is not applied, satisfactory results may, however, be obtained.

INTRODUCTION

The possibility of preserving fixed material dry has, as far as the present author is aware, been neither reported nor attempted previously. In certain cases, however, such a method would be advantageous. It is especially difficult to preserve fixed material in solution on long expeditions in difficult conditions. A quantity of material is not easy to handle and glass bottles are apt to break. The drying of fixed material removes these difficulties. Even a large quantity of material occupies a limited space and the collecting possibilities are thus increased. Dry material is also advantageous to send by post, since much material may be sent by air mail at small cost.

The present idea arose when a part of my material, fixed in summer

1944 and preserved in 70% alcohol, happened to dry. As some of the material could not be replaced, I tried to determine whether buds and root tips which had been dry for 4½ years could not still be used. Since the results seemed promising, the dry-preservation method has been applied to some additional material using different fixatives and stains.

Methods

The original material, which had been dry for 4½ years, contained buds of *Fumaria officinalis*, fixed in the CRAF-solution (Webber's modification), and root tips of *Rubus humulifolius*, fixed in Fleming-Benda. Both these species have small chromosomes.

The dried material was put into 10% alcohol for two days to swell. After two hours in 50% alcohol the material was embedded in paraffin according to the usual method. The sectioning presented no difficulties. It seems, however, to be best to use some method which softens the tissues, e.g. embedding with butyl alcohol.

The chromosomes were stained with iodine gentian-violet. In *Fumaria officinalis* the treatment used (15 min. in 0.5% gentian-violet) gave an even more intense staining of the chromosomes than normally. In *Rubus humulifolius* the time for staining had to be extended to 4–5 hours. It is possible that fixatives containing much osmic acid render staining after a long desiccation more difficult. This is also indicated by the fact that the bleaching of the slides in perhydrol takes a longer time than usual.

In Fig. 1, 2 and 3 are shown camera lucida drawings representing the 16 meiotic chromosomes of *Fumaria officinalis*[1] and the 14 mitotic chromosomes of *Rubus humulifolius*. The chromosomes and their morphology are as distinct as in normal material. A long dry preservation gives the cytoplasm and the cell walls a relatively intense yellow color which does not, however, interfere with the study of chromosomes.

In addition to the above "spontaneous" material a few experiments have been made using root tips of *Pisum arvense* and *Allium Cepa*. The roots of the former species were fixed with the CRAF-solution (Randolph's modification) and with 2BD. After fixation the roots were transferred to 70% alcohol (three changes, ½ hour in the last). Thereafter the roots were kept absolutely dry for one month. After this they were swollen in the manner described above.

The material fixed in CRAF as well as in 2BD was stained either with gentian-violet or Feulgen (schedules according to Darlington

[1]See VAARAMA, A. 1949. The chromosome number of *Fumaria officinalis*. Hereditas, 35, 251–2.

and La Cour,)[2]. Both gave good results. Fig. 4 shows a root tip mitosis in *Pisum arvense* (CRAF combined with iodine-gentian-violet). The morphology of the chromosomes has obviously not suffered during the dry-preservation, e.g. the satellites are clearly discernible.

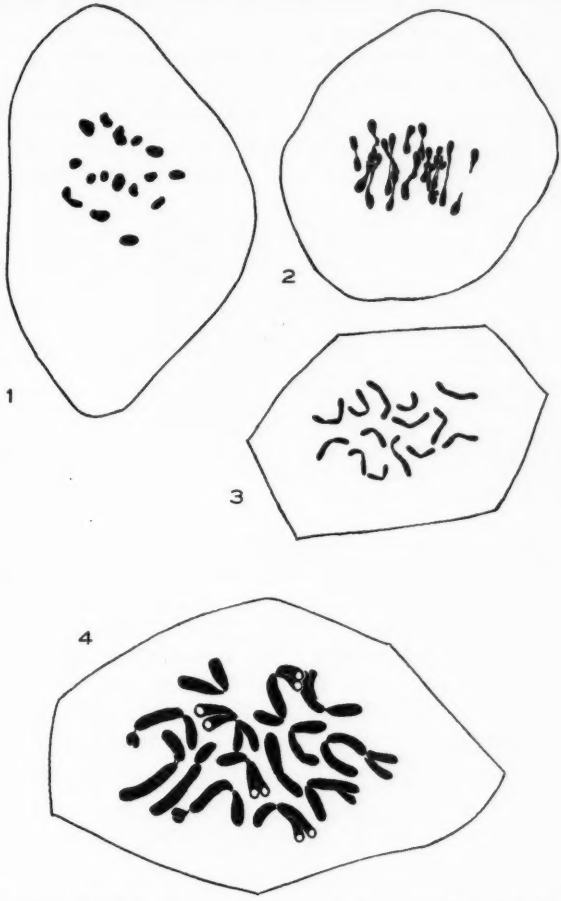


FIG. 1–3.—Fig. 1 and 2. Meiotic metaphase in *Fumaria officinalis* with 16 bivalents. Dry-preserved 4½ years, CRAF fixation, iodine-gentian-violet staining.—Fig 3. *Rubus humulifolius*. A root tip metaphase with 14 chromosomes. Preservation and staining as above. Fleming-Benda fixation.—×2900.

FIG. 4.—*Pisum arvense*. A root tip mitosis with 14 chromosomes. Dry-preserved one month, CRAF fixation, iodine-gentian-violet staining.—×2900.

[2]DARLINGTON, C. D., and LA COUR, L. 1947. The Handling of Chromosomes. *2nd Ed.*, Allen and Unwin, London.

The dried roots may thus in addition to chromosome counts be used for chromosome morphology studies.

The suitability of the dried roots for squash preparations was also tried. *Allium Cepa* material which had been fixed in aceto-alcohol (2 hours in 1:3 solution) and thereafter kept dry for one month was used for squash preparations made either with aceto-orcein or with Feulgen (schedules according to Darlington and La Cour)[2]. The cover glass was separated in 70% alcohol and the slides were made permanent with Euparal. The chromosomes were stained satisfactorily. It turned out, however, that the dry-preserved chromosomes had become fragile and were therefore fragmented and crum-

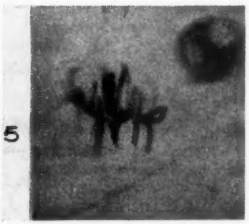


FIG. 5.—*Allium Cepa*. A root tip mitosis in side view. Dry-preserved one month. Aceto-alcohol fixation, aceto-orcein squash preparation applying only slight pressure. —×1000.

bled owing to the pressure applied when they were squashed. Consequently slight pressure was used, the fragmentation thus being avoided. The preparations made according to this method were fairly satisfactory (Fig. 5). It is, however, obvious that dried material is not especially well suited for squash-methods.

For the foregoing experiments only plant material has been used. It seems probable that this method cannot be applied to animal material. Neither does it seem to be good for quite tiny plant roots, as these are difficult to handle when fragile.

LABORATORY HINTS
FROM THE LITERATURE

DEPARTMENT DEVOTED TO ABSTRACTS OF BOOKS AND PAPERS FROM OTHER JOURNALS DEALING WITH STAINS AND MICROSCOPIC TECHNIC IN GENERAL

MICROSCOPIC AND OTHER APPARATUS

HANNA, G. D. **Comments regarding the McArthur inverted microscope.** *J. Roy. Micr. Soc.*, **69**, 86–7. 1949.

An abstract of the original article appeared in Stain Techn. **23**, 155. The present comments are criticisms for improvement of this remarkable instrument. The first comment deals with material for construction. It is suggested that alloys of aluminum or magnesium be used. The second comment deals with the angle of the ocular, suggesting a 15° incline from the upright. Coating the right angle prism with magnesium fluoride or cryolite is suggested in the third place. The author continues with the idea that a penta prism or an optically flat, first surface mirror be used. Further discussion of the problem of complete inversion of the image constitutes the rest of this short but stimulating article.—*Paul G. Roofe.*

HUGHES, A. F. W. **The technique of ciné-photomicrography of living cells.** *J. Roy. Micr. Soc.*, **69**, 53–64. 1949.

This is an excellent account of ciné-photomicrography applied to the phase-contrast microscope in the study of living cells, chiefly with tissue cultures. Complete photographic technic is given. The apparatus is adequately described. This description includes an excellent diagram illustrating the focusing unit which is attached to the camera. There are two plates: one illustrating the instrument in seven different views; the other contains views of living cells in four different exposures. A circuit diagram is included. A full account of the processing of the film is added. A short summary completes the article.—*Paul G. Roofe.*

SARTORY, P. K. **The effects of cover glass thickness on the spherical corrections of objectives.** *The Microscope*, **7**, 122–6. 1949.

The use of cover glasses thicker than computed for in front of an objective produces spherical over-correction. Lowering of the objective will produce fog around the image while raising the objective produces a ring. Too thin a cover glass will produce spherical under-correction and therefore lowering the objective will produce a ring around the object while raising the objective will produce fog. The objective will be approximately spherically correct when raising or lowering the system produces the least difference between the upper and the lower out-of-focus images. Shortening of the draw-tube of the microscope will bring about a correction for over-corrected objectives and lengthening of the draw-tube a correction for under-corrected objectives.—*G. Crossman.*

TAYLOR, E. WILFRED. **The phase-contrast microscope with particular reference to vertical incident illumination.** *J. Roy. Micr. Soc.*, **69**, 49–52. 1949.

This article is an excellent account of the phase contrast principle applied to use with reference to vertical incident illumination. A complete description is given of the apparatus used in this procedure, with a diagram illustrating the lamp unit, projector unit and the microscope equipped with phase contrast apparatus and incident light illuminator; the latter is figured in an excellent photograph. Complete details are presented for adjustments necessary in the operation of the equipment. The final portion deals with the interpretation of the image, illustrated by twelve plates, picturing the surfaces of the following: (1) white-heart malleable iron; (2) grey iron; (3) high-speed tool steel; (4) case-hardened mild steel; (5) zinc; (6) aluminum.

In this interpretation, considerable emphasis is placed on the difference between ordinary incident illumination and contrast phase incident illumination.—*Paul G. Roofe.*

PHOTOMICROGRAPHY

THIERY, G. **L'emploi d'artifices photographiques en technique microphotographique: le "bas-relief", la "solarisation".** *Bull. Hist. Appl.*, **26**, 22–5. 1949.

The writer mentions the failure of conventional photomicrography to bring out certain important details, and calls attention to two artifices which can be used to emphasize some features not brought out in ordinary photography. The first is the "bas-relief" method, in which a print is obtained by exposure to a superimposed negative and positive (the latter slightly less intense than the former), one slightly off-center with respect to the other; the bas-relief effect is of course entirely artificial, but it does serve to bring out details of the specimen not ordinarily shown. The second method is that of "solarization", in which a developed but unfixed negative is briefly exposed to light before fixation; the exposure is of 1–4 sec. to a 100 W light 1 m. away from the negative, using white light in the case of orthochromatic emulsion, red light in the case of panchromatic emulsion. A print made from such a negative has modified tone values: whites remain white, light grays remain gray, but dark grays become black and blacks become white. The result of this latter method is to furnish a picture which somewhat resembles an outline drawing. The article is accompanied by three illustrations to show how prints by each of these two suggested procedures compare with a conventional photomicrograph of the same section.—*H. J. Conn.*

DYES AND THEIR BIOLOGICAL USES

GATENBY, J. BRONTE, and MOUSSA, TOHAMY A. A. **The Sudan black B technique in cytology.** *J. Roy. Micr. Soc.*, **69**, 72–5. 1949.

From Lison's "Histochimie animale" (1936), the authors use the suggestion that Sudan black B may replace Sudan III or IV as a cytological stain. The dye was a product of I. G. Farbenindustrie, and as Baker (1944) has shown, may take the place of silver and osmic acid methods in staining cytoplasmic inclusions. As to its use as a "universal stain", one cannot predict. A schedule for staining is given for thin smears on cover slips. A discussion is presented concerning the sudanophility of various organelles, especially the Golgi apparatus.—*Paul G. Roofe.*

ANIMAL MICROTECHNIC

CAMPBELL, A. C. P. **The histological preparation of bone-marrow particles, uterine curettings and other small tissue fragments.** *J. Path. and Bact.*, **60**, 633–4. 1948.

The author recommends the following procedure: Aspirate 2–3 ml. of blood and discharge it into a small test tube containing a small amount of heparin powder, and mix gently. Transfer with a Pasteur pipette to a large Petri dish and spread out. Pick out tiny marrow flecks with a Pasteur pipette for smearing, and transfer the rest of the flecks to a small glass container. Remove as much blood as possible from the amount transferred with the flecks. Pipette fixative (mixture of 4 parts 10% formalin in saturated aqueous $HgCl_2$ + 1 part methanol) into the container and fix for 4–6 hr. Pipette off fixative. Add water and wash by gentle agitation for a few sec. Pipette off most of the water and transfer the residual drop or two containing the marrow fragments to a slide. Remove excess water from the slide with filter paper. Push the marrow fragments together into a compact group and place a glass ring around them. Melt a tube of 2 or 2.5% agar, cool to 50°C., and pipette the agar into the ring to form a layer 5–6 mm. above and surrounding the tissue fragments. Warm the slide first to ensure even penetration by the agar. Allow the agar to set. Wet the slide, slide off the glass ring, and push out the agar block. Trim the block. This block can now be dehydrated, cleared, infiltrated with paraffin, and sections cut as usual.

For uterine curettings and other larger fragments, a large glass mold is necessary.—*S. H. Hutner.*

ENDICOTT, K. M., GILLMAN, T., BRECHER, G., NESS, A. T., CLARKE, F. A., and ADAMIK, E. R. **A study of histochemical iron using tracer methods.** *J. Lab. and Clin. Med.*, **34**, 414–21. 1949.

Combined histochemical, radioautographic, and tracer methods were used to study the absorption and distribution of single test meals of radioiron, in guinea pigs, rats and one dog. The iron demonstrated histochemically in the duodenal mucosa, the mesenteric and cervical lymph glands, liver, and spleen was derived largely from other sources than the single test meal.—*John T. Myers.*

HENNEMAN, PHILIP H., WEXLER, HILDA, and WESTENHAVER, MARY M. **A comparison of eosin-acetone and phloxine-propylene glycol diluents in eosinophile counts.** *J. Lab. and Clin. Med.*, **34**, 1017–20. 1949.

The circulating eosinophile count by the chamber method was studied using two different diluents. With an acetone-eosin mixture (2% aqueous, 5.0 ml., acetone, 5.0 ml., and distilled water, 90.0 ml.) there is a large and rapid decrease in cell count with passage of time after dilution of the blood and with aging of oxalated blood. A phloxine diluent (phloxine, 0.05 g., propylene glycol, 50.0 ml., and distilled water, 50.0 ml.) gave more consistent results with no evidence of destruction of eosinophiles.—*John T. Myers.*

MACKLIN, CHARLES C. **The two types of epithelium of the finest bronchioles of the albino mouse as revealed by supravital silverization.** *Canad. J. Res., sec. D*, **27**, 50–8. 1949.

To learn more about the epithelium of the last quarter millimeter of the purely conducting bronchioles, healthy albino mice were examined by the following method: anesthetize lightly with ether; pinch up the diaphram carefully and nick it; with a hypodermic syringe inserted into the exposed trachea fill the collapsed lungs to about the stage of full inspiration with an ammoniacal silver solution; drain after two minutes and refill with 10% formalin; tie off trachea; remove heart and lungs and place in 10% formalin for a day or more exposed to daylight and at times to direct sunlight; cut frozen and paraffin sections. The ammoniacal silver solution is prepared as follows: (Solution 1) dissolve 5 g. $AgNO_3$ in 300 ml. distilled water; add dilute *aqua ammoniae* until the precipitate found is nearly but not entirely redissolved; filter and add water to make 500 ml.; (Solution 2) dissolve 1 g. $AgNO_3$ in a small quantity of water; pour into one-half liter boiling water; add 0.83 g. Rochelle salt dissolved in a small quantity of water; boil for half an hour until a gray precipitate collects as a powder in the bottom of the flask; filter hot; add water to make 500 ml.; keep the two solutions in the dark; mix equal parts when ready to use. By this method two types of epithelial cells were differentiated. One type is ciliated. The unciliated type is much more conspicuous but its morphology and function are not well understood.—*H. P. Riley.*

NICOL, J. A. COLIN. **The Weigert-Pal method for paraffin-embedded material. I. Bulk staining and staining of paraffin sections.** *Canad. J. Res., sec. D*, **27**, 1–3. 1949.

In the Weigert method for staining myelin sheaths of nerve fibers paraffin sections frequently become detached from the slides because of the long immersion in the staining solutions. To avoid this the following methods were used: Fix hyomandibular nerve and spinal cord of the ratfish (*Hydrolagus collieri*) and the tibial and peroneal nerves of the white rat for two days in 10% formol (4% formaldehyde) neutralized by powdered chalk; place in 2.5% potassium bichromate at 37°C. for 12 days; transfer to solution of gliabeze (2 g. chromium fluoride; 5 g. copper acetate; 5 ml. glacial acetic acid; 100 ml. distilled water) at room temperature for 48 hr.; wash 24 hr. in running tap water; dehydrate in alcohols; clear in cedarwood oil; embed in paraffin with 56° melting point; section at 15 to 20 μ; remove paraffin; coat sections with celloidin; hydrate; stain in Kulschitzky's acid hematoxylin (dissolve 1 g. hematoxylin in 10 ml. 95% ethanol; add 88 ml. distilled water and 2 ml. glacial acetic acid; add 0.1 g. sodium iodate to ripen; filter) at 37°C. for 24 hr.; wash 30 min. in running tap water; differentiate by Pal's method of alternate baths of potassium permanganate and oxalic acid-sodium sulfite solution; wash 1 hr. in running tap water; mount directly or stain

30 sec. in 1% aqueous safranin; wash 5 min. in running tap water; dehydrate in two changes of dioxane for 5 and 2 min.; clear in xylene; mount. The following technic may be substituted in staining with hematoxylin: section; float section on surface of hematoxylin solution for 24 hr. at 37°C.; remove to large volume of tap water; mount sections on slide; remove paraffin; hydrate and differentiate; counterstain and mount as above. Material may also be prepared by the following method of bulk staining: mordant in potassium bichromate and gliabeze as above; wash; place in Kulschitzky's hematoxylin for 24 hr. and 48 hr. at 37°C.; wash 5 hr. in tap water; embed and section as above; mount; remove paraffin; hydrate; differentiate. By these methods myelin sheaths appear dark blue-black against a colorless or red background of connective tissue.—*H. P. Riley.*

WANG, K. J. and GROSSMAN, M. I. **A simplified technique for the preparation of sections by freezing and drying.** *J. Lab. and Clin. Med.*, **34**, 292–6. 1949.

The recommended procedure is as follows: Freeze the tissue in liquid pentane which has been cooled with liquid air (in most cases CO_2 and acetone will do). Connect a Cenco Supervac pump of the mercury vapor diffusion type, operating by application of heat to the mercury in the boiler, to a Cenco Hyvac pump by means of a glass tube. Use a pyrex tube surrounded by a Dewar flask as a dehydration chamber, Drierite being placed in the bottom of the tube. Also place Drierite in the connecting tube between the dehydration chamber and the pump, where is also placed a freezing trap surrounded by a mixture of solid CO_2 and acetone within a wide mouth Dewar flask. Wrap the joints with para rubber tape and cover with shellac. Fill the Dewar flask surrounding the dehydration chamber with dry ice and dimethoxytetraglycol. This maintains a constant temperature of 31° C. With the pumps running constantly, dehydration requires about 5 days. Then turn off the heater on the mercury pump, and stop the forepump in about 30 minutes. Embed in paraffin and cut in the usual way. Thin and excellent sections may be obtained.—*John T. Myers.*

PLANT MICROTECHNIC

ARNASON, T. J., and WALKER, G. W. R. **An irreversible gene-induced plastid mutation.** *Canad. J. Res., sec. C*, **27**, 172–8. 1949.

Studies in variegated barley indicate that green, variegated and albino types result from the maternal inheritance of plastids. In the presence of certain nuclear genes green plastids may continually mutate to albino. Plastids were studied by preparing freehand sections of variegated leaves by a modification of Froiter's method and examining sections freshly cut at 20–50 μ thick in a water mount. When a small drop of iodine was added to the preparation the visibility of the plastids was increased. In green mesophyll cells, 37 to 46 green plastids 5–7 μ in diameter were present; in a transition zone between green and white tissues only 1 to 21 were present and some were reduced in size; in these cells 28 to 36 small bodies with a diameter of 1–3.5 μ were also present and these granules stained the same as normal plastids with iodine; in albino cells, no green plastids were found but 40 to 50 of the small granules were present. These granules are believed to be mutated plastids; they enlarge and deposit starch when white leaves are floated for 24 hr. in 10% cane sugar solution.—*H. P. Riley.*

BROWN, R., and RICKLESS, P. **A new method for the study of cell division and cell extension with some preliminary observations on the effect of temperature and of nutrients.** *Proc. Roy. Soc.*, **B, 136**, 110–25. 1949.

A novel technic for determining the total and the meristematic number of cells in a root meristem is described; it involves macerating the tissue in a known volume of fluid, and subsequently determining the appropriate number of cells in an aliquot on a haemacytometer slide. Root tips are cultured in batches on sintered glass disks with different nutrient media and at four temperatures. Samples of four roots are taken from each culture at intervals of 12 hr., and with each sample the average total number, the average meristematic number of cells, and the average length of each root are determined. From these primary data rates of division and indices of extension are calculated.

It is shown that no division occurs in the absence of sugar, and the rate of division is increased by providing inorganic salts with the sugar, and still further increased by supplying yeast extract. With each nutrient the rate of division is higher at 15° than at 25°C., but lower at 5° than at 15°C. No extension occurs in the absence of sugar, and the greatest extension is given with sugar and inorganic salts. Yeast extract in the form and concentration used in these experiments tends to depress extension.—*T. E. Weier.*

MICROÖRGANISMS

KNOTHE, H., MAASEN, W. **Über eine Modifikation der Gramfärbung mit Borsäure als Vorbeize and Pikrinsäure-Sublimat-Natriumacetat-Wasser-Gemish statt Lugolscher Lösung als Hauptbeize.** *Zts. Hyg. Infektionskrankh.*, **128, 402–5. 1948.**

A modification of the Gram stain is given which is said to give the same tinctorial results as the original procedure. Phenol is included in the 10% alcoholic gentian violet stain; and instead of Lugol solution, an aqueous solution of 1% picric acid, 5% $HgCl_2$, and 0.4% sodium acetate is used as a mordant.—*Ivan Saphra.* (*Courtesy of Biological Abstracts.*)

LOPES DE FARA, J. 1949. **Valôr do método Faraco para coloraçao do bacilo de Hansen em cortes.** *Rev. Brasileira Leprologia*, **17**, 18–26.

Faraco's method (published in 1938) involves treatment of tissue sections with mineral oil before staining by the Ziehl-Neelsen technic. The advantages are: the larger number of bacteria stained; the greater number of positive diagnoses in questionable sections with few bacteria; and the possibility of demonstrating acid-fast organisms in tissue embedded in paraffin for as long as 5 years.—*G. A. Emerson* (*courtesy of Biological Abstracts.*)

MORSE, ROY E., and ESSELEN, WILLIAM B. Jr. **Triphenyl-tetrazolium chloride as a vital stain for dried bakers' yeast.** *Food Res.*, **14, 123–9. 1949.**

The use of triphenyl-tetrazolium chloride as a microscopic vital stain shows that live and dead yeast cells reduce the dye to a red color of equal intensity. The resulting stain is largely peripheral in nature, with occasional center staining. Unidentified long bacterial rods reduce the dye to a bright-red color, indicating the possibility of using triphenyl-tetrazolium chloride as a bacteriological stain.

Of the macroscopic methods employed, the following one, using the reflection meter to measure difference in color development as related to yeast viability, was found to give the best results:

(1) Prepare a suspension of dried yeast to be examined for viability by making 3 g. up to 100 ml., using distilled water, 40°C. For compressed yeast use a 10-g. sample. Prepare a similar suspension using yeast of known viability of identical moisture or corrected moisture content. (2) Mix 49 ml. of the above suspension with 1 ml. of a 1% aqueous solution of triphenyl-tetrazolium chloride. Incubate the thoroughly mixed samples for 30 minutes at 40°C. (3) Using a reflection meter, read the developed color by the suppressed zero method. For establishing the proper range, use Munsell Color 2.5R-4/10 to adjust the instrument to zero, and 5R-5/12 to adjust it to 10. Mix suspension thoroughly before filling the test solution into a 30 ml. optically flat cuvette, such as Wilkens-Anderson Company, 7103-RCK. Read immediately after pouring. For making a reading, invert the search unit of the instrument and completely cover the light source with the test sample. (4) Compare the readings of the yeast of known and unknown viability. If the unknown has a lower reading, it has higher viability.—*William G. Walter.*

POTTY, G. L. **A simplified procedure for staining acid-fast bacteria in smears and tissue sections.** *Bul. Internat. Assoc. Med. Mus.*, **28, 188–91. 1948.**

The author proposes a method which can be used for either smears or paraffin sections carried down to water as usual. The following two solutions are employed: (1) Dissolve 4 g. certified basic fuchsin in 25 ml. 95% ethyl alcohol, and 12 g. liquified phenol crystals, mix well, and add 25 ml. glycerol C.P. and distilled water q.s.ad 130 ml., let stand 30 min., and pass through a diatomaceous filter

or Seitz filter sheet. (2) Prepare 110 ml. 2% aq. certified malachite green; mix with 15 ml. glacial acetic acid, and then add 25 ml. glycerol, C.P. (Both solutions keep indefinitely at room temperature.) The technic is as follows: Stain sections 5 min. or smears 3 min. in solution 1; wash in distilled water. Decolorize and stain simultaneously in the acid malachite green solution, 5 min. for sections, 2 min. for smears (restaining the smears 1–2 min. if background is not an even green); dehydrate, clear, and mount.—*Ethel Lieb.* (*Courtesy of Biological Abstracts*).

HISTOCHEMISTRY

ALBERT, S., and LEBLOND, C. P. **Age changes revealed by carbonyl reagents in tissue sections.** J. Anat., **83**, 183–94. 1949.

The carbonyl reagents (fuchsin sulphurous acid and dinitrophenylhydrazine) were used as histochemical stains to determine age differences in tissues of pedigreed mice. The carbonyls appear to be aldehydic lipids held in acetal linkage. Steroid-secreting cells stain more intensely than others. Intensity of staining of kidney, endocrine glands, sex glands, and accessory sex organs increases until puberty, decreases later. For staining with FSA after formalin fixation, the authors conclude that some oxidation, either from the air, as by standing in water in a shallow dish overnight, or by addition of 1–2 drops of 3% H_2O_2 (Merck, U.S.P.) per ml. of tap water and washing in this for ½ hour, is required. These histochemical stains can be used as tests of function if caution is exercised in the interpretation of results.—*Warren Andrew.*

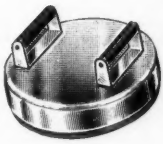
PANIJEL, JACQUES. **Effets de sels biliaires sur certains complexes protéiques Gram-positifs.** *Bull. Soc. Chim. Biol.*, **30**, 336–46. 1948.

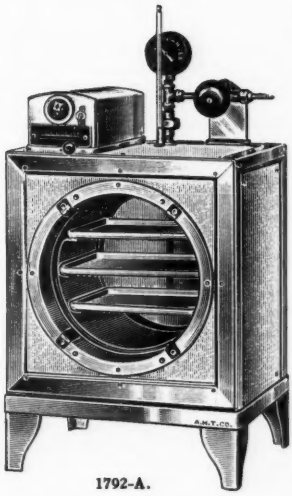
Bile salts are known to be capable of removing from the Gram-positive bacteria the ribonucleic-acid component of a protein complex which is responsible for the positive Gram-staining. The ribonucleic acid can be brought to reunite with the protein, thus restoring the positive Gram-staining. It is shown that this effect is not due to the surface activity of the bile salts, since it is found to be possible to inhibit the growth of bacterial cultures by very low concentrations of bile salts in a medium in which the surface tension has been decreased by a detergent, in such a way that the bile salts alone or the detergent alone are unable to produce any effects. Under these conditions the magnitude of the bacteriostatic effect seems to be parallel to the power of suppressing the Gram-staining. The work was carried on with *Staphylococcus aureus.*—*Gunnar Steensholt* (*Courtesy of Biological Abstracts.*)

PARAFFIN INFILTRATING AND EMBEDDING OVEN

VACUUM TYPE

PARAFFIN INFILTRATING AND EMBEDDING OVEN, Vacuum Type, Electric, A.H.T. Co. Specification. For the rapid infiltration of specimens which, because of their consistency, density, cavity structure or size, infiltrate with difficulty by the usual methods; see R. D. Lillie, Archives of Pathology, Vol. 16, No. 2 (August 1933), pp. 232–235. Tissue damage or shrinkage caused by prolonged exposure to embedding temperatures can be minimized by use of this method, which is particularly useful when specimens have many internal cavities.





1792-A.

Range 20 to 70°C (68 to 150°F), with temperature setting scale and pointer for convenient adjustment. Vacuum chamber is cylindrical, 9¼ inches deep × 9¼ inches diameter, made of pressed steel with nickel plated finish, built into a substantial anhydric insulated jacket of hard asbestos board with Stainless steel frame, and is provided with thermometer well and three removable shelves.

Operating Sensitivity, i.e. maximum variation in temperature at location of thermometer bulb, ±0.5° C.

Operating Uniformity, i.e. maximum variation in temperature within the working space of the oven in relation to the temperature at the thermometer bulb, approximately ±1.5°C.

The chamber is sealed by means of a lead gasket in the removable front door. Final sealing is simply and quickly accomplished by slight tapping with a wooden mallet. Three shelves are supplied with each oven; shelves are all 7½ inches deep, and 7¾, 8 and 9 inches wide, respectively, to fit the cylindrical interior. Overall dimensions, including vacuum gauge and door handles, 26 inches high × 14¾ inches wide × 14 inches deep.

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STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stains approved since those listed in the last number of this Journal.

STAINS CERTIFIED SEPT. 1, 1949 TO NOV. 30, 1949*

Name of dye	Certification No. of batch	Dye Content	Objects of tests made by Commission†	Date approved
Brilliant green	DBg-4	95%	Bacteriology (in media)	Nov. 8, 1949
Crystal violet	CC-17	88%	Histology; cytology; bacteriology (staining; in media)	Nov. 22, 1949
Ethyl eosin	NEe-8	77%	Histology	Oct. 12, 1949
Fuchsin, basic	DF-12	99%	Bacteriology (in media)	Nov. 3, 1949
Fuchsin, basic	CF-36	93%	Histology; Feulgen reaction; bacteriology (staining; in media)	Nov. 16, 1949
Methyl orange	LM-3	90%	Histology	Sept. 1, 1949
Methylene blue chloride	LA-19	89%	Histology; bacteriology (staining); compounding of blood stains	Nov. 29, 1949
Methylene blue chloride	NA-30	85%	Ditto	Nov. 29, 1949
Nile blue A	CNb-3	89%	Fat staining	Oct. 19, 1949
Phloxine B	CPh-6	89%	Histology	Oct. 12, 1949
Wright stain	CWr-22	——	Blood staining	Nov. 22, 1949

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seems to give the most severe check to its staining value. Certification does not in any instance, however, imply approval for medicinal use.

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Managing Editor: Curt Stern, University of California

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